VOL. LXXVII, NO. 768 JANUARY-FEBRUARY, 1943

THE AMERICAN NATURALIST

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A BI-MONTHLY JOURNAL

Devoted to the Advancement of the Biological Sciences with Special Reference to the Factors of Evolution

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THE SCIENCE PRESS

Lancaster, Pennsylvania

The American Naturalist

Established in 1867

Edited by J. McKEEN CATTELL and JAQUES CATTELL

Manuscripts intended for publication should be sent to the editors of THE AMERICAN NATURALIST, The Science Press, Lancaster, Pennsylvania.

Short articles containing summaries of research work bearing on problems of genetics, heredity and organic evolution are especially welcome, and will be given preference in publication.

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THE SCIENCE PRESS

Entered as second-class matter, March 19, 1908, at the Post Office at Lancaster, Pa., U. S. A., under the Act of Congress of March 3, 1879.

THE AMERICAN NATURALIST

THE SCIENCE PRESS PRINTING COMPANY
LANCASTER, PENNSYLVANIA

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AMERICAN NATURALIST

EDITED BY J. MCKEEN CATTELL AND JAQUES CATTELL

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LANCASTER, PA.
THE SCIENCE PRESS
1943

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AMERICAN NATURALIST

VOL. LXXVII *January–February, 1943* No. 768

EDMUND B. WILSON—AN APPRECIATION

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WITH the passing of Edmund B. Wilson on March 3, 1939, at the age of eighty-three years, we biologists have lost him who long was our most beloved, revered and helpful guide and comrade in the great mutual adventure of exploring the world that is buried deep within ourselves and all living things. As leader, inspirer and teacher he was preëminent, as the many who were directly and profoundly influenced by him will attest. While many biologists were disputing whether chromosomes were permanent entities at all, he and Stevens provided the first definite and proved examples—those concerned with sex itself—of chromosomal inheritance. This work, taken in connection with the general theory of sex determination which Wilson based upon it, and with the correlative studies which he made on other chromosomes, marked the establishment of what we now call "cytogenetics," and therewith placed genetics in general on solid, visible ground.

Both in the prior preparation of the ground for cytogenetics and in the further elaboration of the theories erected on it, Wilson played a major role. His own observations, experiments and analyses supplied much of the factual and theoretical material required at the most critical points. His systematic work—carried on for over half a century—of evaluation, selection, coordination, his amazing recrystallization and ordering of the whole intricate mass of extant data and hypotheses deal-

ing either directly or indirectly with the all-inclusive problem of how the entire individual may lie implicit in the single cell, all this has fully stood the test of time and is an accepted and basic part of all modern theory in genetics, ontogenesis and related subjects.

Wilson's contact with science began when the establishment of the theory of evolution and even of the cell theory in its primitive form were still fresh in men's minds, and when these doctrines were actively serving for the reinvestigation or reassessment of all known biological facts. Mitosis and the chromosomes had not yet been discovered, nor even the fact that fertilization involves a union of two nuclei, one from each parent. For oil-immersion lenses and Abbé condensers, anilin dyes and section cutting had only just been invented. And experiment had hardly entered the fields of morphogenesis, cell study or evolution. As for the teaching of biology, it and other sciences were practically unknown in the schools and even in most of the smaller colleges (see Wilson, "Teaching and Research in the Natural Sciences," 1909). Thus Wilson's life spans practically the entire period of growth, not only of genetics, but of biology in general as we know it. Moreover, his own scientific activities largely illustrate this growth, for as improved methods of approach—either those of hand or brain—arose, Wilson time and again was to be found among the vanguard of those adopting them.

Although the childhood of "Eddy," as his parents called him, was spent in a small town—Geneva—on the prairies of Illinois, his home environment was conducive to the development of intellectual interests. For the parents of both his father, Federal Judge Isaac C. Wilson, and of his mother, Caroline Clarke Wilson, had brought with them from New England a tradition of education, general culture and music—without, however, any special background of science. And it is probably significant that many of his ancestors were sea-faring men of the old New England school, with their spirit of adventure, of independence and, above all perhaps, of skilful craftsman-

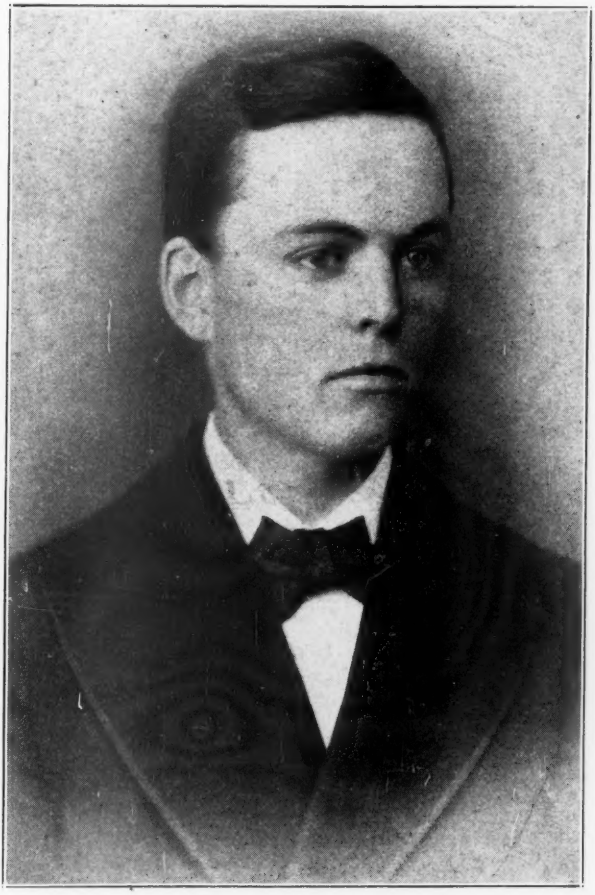
ship. Eddy's parents, as well as the Pattens—the childless aunt and uncle at whose house he spent much time—were exceptionally sympathetic and understanding. With his elder brother and his younger brother and sister he spent a happy and wholesome childhood. He early showed his scientific bent, particularly in the form of an intense interest in animals, and his artistic bent, which was directed especially towards music. His elders did not discourage these interests. At six he was already tending small animals he had caught; he kept them in the room set aside for him at the Pattens, who drew the line only at manure beetles. At sixteen, after a strenuous year's experience teaching a country school, he was already eager to devote his life to biology. The path ahead was by no means free from material difficulties, but by working summers as well as after hours at various jobs, by winning fellowships and by the aid of an occasional advance from his elder brother, Eddy managed to gain the education that he sought.

After his year as a school teacher, Wilson entered upon a period of increasingly good preparation, with particular attention to biology, chemistry and other sciences, at various colleges in turn. To most of these he was drawn by the attractive accounts of them sent by his elder cousin, Sam Clarke, who, likewise having biological leanings, had preceded him there. The first college year he spent at Antioch in Ohio, then a year at Chicago in preparation for the Sheffield Scientific School of Yale, where he spent three years. At Yale he studied under the naturalist Verrill and, on the side, attended very stimulating lectures on heredity and evolution given by the stock-breeder Brewer. As a thesis for the Ph.B. degree he wrote two descriptive papers on the Pycnogonids ("sea spiders"). During his last year there, in 1878–9, he saw for the first time a picture of mitosis, in a work just published by Mark on fertilization and cleavage in the snail's egg. This proved to be the beginning of Wilson's permanent interest in these matters.

After this he was delighted to secure a fellowship at the great graduate school of science that had not long before been established at Hopkins. Here, chiefly under the influence of the magnetic and wide-visioned zoologist Brooks and of Huxley's former associate, the physiologist Martin, Wilson became accustomed to seeing beyond and beneath the circumscribed domain of traditional natural history. From now on he began to fix, as his central objective, upon the problem of how the individual lies determined within the germ. As he later (in "The Cell," 1896) expressed the matter, "that a cell can carry with it the sum total of the heritage of the species, that it can in the course of a few days or weeks give rise to a mollusk or a man is the greatest marvel of biological science." In a sense, all the work which he subsequently did was in one way or another concerned with this question, in which both embryology and genetics lie epitomized.

Laying the Embryological Groundwork

In the years of Wilson's graduate work (1878–1881) and for some time thereafter the best available means of approach to this recondite problem appeared to lie in the accurate descriptive study of development. This was a field in which much remained to be explored and which still seemed to hold promise of revealing many mysteries, both concerning the present relation of the individual to his germ and concerning the way in which, through past ages, germ and individual have come to be what they are. In this field, in his doctor's thesis giving the results of three summers' studies of the development of the colonial polyp *Renilla,* he did a distinguished piece of work, of highly critical and analytical character, using the technique, which had only just been invented, of section cutting. Among other things, he showed that, despite the regular division of the nuclei, the cytoplasmic cleavage of the egg of this form is very variable, either segmenting the egg-surface definitely from the beginning or being



Wilson at eighteen, in his natural history days. At this time, 1875, he was an undergraduate at the Sheffield Scientific School of Yale.

more or less unexpressed until even the fourth division, when simultaneous demarkation of all the cells may occur; similarly variable is the spatial pattern of the cytoplasmic cleavage. In tracing the origin of muscle cells, spicules, mesogloea, etc., the absence of a separate mesoderm layer was confirmed. Interesting physiological, phylogenetic and ontogenetic conclusions were also drawn from the observations on the origin and nature of the different members of the colony. Shortly after the manuscript was completed, when Wilson was in Europe (1881–83), it won the commendation of Huxley, who introduced Wilson's presentation of it to the Royal Society.

In order to follow up Wilson's activities along this line in a consecutive way, we may for the moment pass over these two years in Europe, as well as the next two, spent mainly in teaching and writing, at Williams College (1883–84) and the Massachusetts Institute of Technology (1884–85). In 1885, when Bryn Mawr College was founded, Wilson secured an appointment as head of the department of biology there. This he held until 1891, when he was called to the department of zoology at Columbia by H. F. Osborn. At Bryn Mawr, thanks to the far-sighted educational policies of the dean, Miss Thomas, conditions for Wilson's research were again favorable. This enabled him now to follow up his work on *Renilla* by a similar study on the earthworm, *Lumbricus* (1887, 1889, 1890).

In his work on earthworm embryology Wilson traced the early stages in detail and succeeded in demonstrating that in this form and probably in annelids in general—contrary to some current reports—the mesoderm is formed by teloblast cells. This finding increased the significance of this feature of development for the determination of homologies and it helped greatly—especially in conjunction with Wilson's next embryological work, on *Nereis*—in indicating that most animals above the simple two-layered ones (Coelenterates) can be divided into two great stems. In one of these, the "teloblast series" (in-

cluding nemertines, flatworms, annelids, arthropods and mollusks), the members having the type of embryology more primitive for this group are characterized by a spiral, "mosaic" cleavage, in which specific cells are early set aside for teloblast cells, from the chainwise division of which the mesoderm is derived, while in the other, or "enteroblast series" (including *Sagitta,* Molluscoids, Echinoderms and Chordates) there is, primitively, a radial cleavage and the mesoderm is derived from pouches of the archenteron.

Wilson's modesty, and his caution in treading the already notoriously boggy ground of phylogenetic questions, prevented his laying emphasis upon the fundamental bifurcation of higher organisms thus indicated, although he called attention to it in the graduate course in zoology which he later gave at Columbia. But the fashion of regarding phylogenetic considerations as unimportant—which was beginning even in the early days of his embryological work, in reaction to the overconfidence shown by the first students of phylogeny—obscures what is probably a very important fact of animal relationships, the significance of which may be better appreciated in the future, with the progress of comparative physiology and biochemistry.

Wilson found the marine annelid *Nereis,* by virtue of the great precision and regularity of its pattern of cell divisions, far better adapted than the earthworm for tracing out the development of all parts in the minutest detail, cell by cell, from the fertilized egg onwards. His classical account of the development of this worm, published in 1890 and 1892, marks him as one of the most outstanding pioneers of "cell-lineage," as this new discipline was called. This study not only cleared up for the first time, in terms of the individual cells concerned, the origin of all the embryological structures in annelids having determinate cleavage. By a wider comparison of these results with those found by others in other types of teloblastic animals, such as flatworms and mollusks, Wilson showed

that while on the one hand there are far-reaching similarities in the whole peculiar cleavage-pattern and in the fate of the different cells of the cleavage stages shown by the widely different forms—understandable only as expressions of very ancient homologies—nevertheless there are also very striking differences between the larger groups, in that some of the parts which are obviously homologous in their later embryos are derived from non-corresponding cells of the early stages, even where these early stages themselves appear alike. In the phylogenetic history, then, one cell must at some time have become substituted for another as the primordium of a given tissue or organ. Thus the cell-lineages, though very valuable guides to homology and phylogeny, could no longer be regarded as absolute criteria, any more than the later stages. As Wilson subsequently emphasized (1894, 1895, 1898), the early stages must be capable of modification like the later, and so, while their remarkable parallelism in such widely differing forms must be ascribed to their long-continued retention of a common ancestral pattern of development (though not to the existence of any common ancestor the adult stage of which was like these embryos), this persistence of the ancient pattern must itself have been conditioned by the continuance of similar conditions of living, to which it was useful for these divers embryos to remain adapted.

In these conclusions Wilson had gradually branched out on to far deeper questions than those of purely descriptive embryology or even phylogeny. His activity in these more analytical fields, in which he was presently to resort to newer, more refined methods of attack, both observational and experimental, had been greatly stimulated by his most exhilarating stay abroad from 1881 to 1883—at Cambridge, Leipzig and especially at Dohrn's marine biological station at Naples. The 26-year-old Wilson had avidly absorbed the newer methods and ideas concerning the cell theory, the evolution theory and biology in general at the centers where they were being most

actively put forward. He had at the same time greatly enriched his life by the international friendships which he formed, by the exotic associations born of his travel experiences and by the rapturous occasions on which he lost and found himself in the transports of music. Such was the attraction to him of the mode of life thus opened up that he twice later, at intervals of approximately ten years, spent the better part of a year in Europe—in 1891–92 at Munich and Naples, and in 1903 again at Naples. From each visit he returned with greater intellectual stature and, conversely, his own influence on the Europeans increased markedly with each visit. His example, moreover, did much to encourage the flow to and from Europe of other young biologists from America, which was then a comparatively backward country in science. It can not be doubted that in the humbleness and in the internationalism of attitude exemplified in Wilson's European visits, in the willingness to learn from scientists in other countries, and in the increased opportunity thus afforded for integration of the best to be found everywhere, the United States owes much of its rise to a place second to none in the field of experimental and theoretical biology. On the other hand, the more intense nationalism prevalent among the scientists of some European countries may in time have acted as a partial brake upon the progress of biology there.

Integrational Work in General Biology

In the two years between Wilson's first stay in Europe and his appointment at Bryn Mawr, having reduced facilities for research, he spent much of his time writing a "General Biology" (1885; second edition, 1895) in cooperation with his colleague, W. T. Sedgwick. This was a work designed to introduce the study of life—both in its animal and plant manifestations—from a more modern and analytical point of view than that hitherto presented to beginning students. The book started with a treatment of the nature of living matter which gave grounds

for inferring that it is composed of the same kinds of atoms as lifeless matter, working according to the same fundamental physico-chemical laws, but giving peculiar results because of its special, complex mode of organization. To quote from the 1885 edition: "For purposes of biological study life must be regarded as a property of a certain kind of compounded matter. But we are forced to regard the properties of compounds as the resultants of the properties of their constituent elements. . . . Reflections of this sort show how ignorant we are of the real properties of the elements and how important a further study of them is [an inference brilliantly vindicated by modern physical findings]. . . . The phenomena of consciousness . . . are not known to occur apart from a living material basis with which they appear to be in some way closely connected" (pp. 5–6). "At every moment of its existence the organism is acted on by its environment; . . . at every moment it reacts upon the environment, maintaining with it a constantly shifting state of equilibrium" (p. 96).

Protoplasm, the cell and cell aggregates (tissues) were then presented on the above basis, that is, neither as static morphological patterns to be memorized nor as pure dynamics floating in a void, but as complexly organized working systems, their morphology and physiology being always considered in the closest relationship with one another. The same procedure was next followed in the detailed study of an example of a multicellular plant, the fern, and of an animal, the earthworm, in such wise that the student might always bear in mind the significance of each feature with reference to the part it played in connection with the other components in the workings of the whole individual and in the maintenance of the species. Everywhere it was shown—but not in the mystical spirit in which this point is sometimes made—that the parts of the organism "are mutually interdependent and . . . the organism as a whole is greater than its parts." Organisms of an intermediate degree of multicellular complexity

had been chosen in order, on the one hand, to avoid confusing the beginning student by excessive complications while, on the other hand, retaining the advantages of early consideration of organs and systems sufficiently specialized to bring the various functions into high relief. The method of presentation adopted made it easy for the student, on comparison of the earthworm and the fern with each other, to see that, far apart though they seemed superficially, practically all their obvious differences were accessory to their one most fundamental difference, that in their mode of nutrition. For a student taking more biology the ground had now been prepared for a comparative view of different forms and for a consideration of the factors that had led them to become different.

The points of view in this book, although not actually novel at the time, need even to-day more emphasis and a wider dissemination than they commonly receive—witness the recurrent recrudescence of vitalistic and other mystical or crude ideas regarding living things. This was, however, much more true in 1885, when biology had only so recently begun to draw away from the animistic preconceptions of mankind in general, and when within its own domains its morphological and physiological sides were still on the whole so blind to one another. Fortunately the book had a wide influence. The 1900 edition can still be used with much profit in an introductory course.

Experimental Analysis of Morphogenesis

As may be gathered from the above evidence of Wilson's interest both in physiology and in comparative morphology, he was one of the few biologists of that time to combine an appreciation of the importance of the experimental and of the comparative observational methods. As he pointed out, the two methods are after all not essentially different, inasmuch as the comparative one makes use of the "experiments carried on by nature," as he termed them in his lecture "Aims and Methods of Study

in Natural History" (1900). "The experiments performed in our laboratories but supplement those that have taken place and are always taking place in nature, and their results must be wrought into the same fabric" (*ibid.*). Both means of approach are, he insisted, necessary and complementary, in view of the complicated organization of present-day organisms, representing as they do the accretion, by natural selection, of so many evolutionary steps. And, as he said later (1914): "What is the living organism, and how has it come to be? We often find it convenient to lay emphasis on one or the other of these questions . . . [but] fundamentally they are inseparable. The existing animal bears the indelible impress of its past; the extinct animal can be comprehended only in the light of the present." Being known to present-day geneticists mainly for his comparative studies on chromosomes, it is not so generally realized by them, however, that in the last pre-Mendelian decade (1890–1900) Wilson had already become one of the foremost in showing the value of experiment in attacking problems concerning the mode of organization of the germ cells. This was in accordance with his belief that "the introduction of experimental methods into morphology is the most momentous step in biological method that has taken place since the introduction of such methods into physiology by Harvey and Haller" (1900, *ibid.*).

The rich possibilities awaiting the use of experiments in this field had hardly been realized until Roux in 1888 started the experimentalist bark on its way. He showed that when blastomeres of the frog's egg are killed the surviving ones (for a time at least) develop "mosaically," *i.e.*, into part of an embryo, as they would have done if in their normal setting. In 1891 Driesch countered by showing that isolated blastomeres of the sea urchin are "totipotent," developing eventually into whole embryos. During Wilson's second stay at Naples (in 1891–92, just before taking up his new position at Columbia) he was plunged into the midst of this startling new line of work

on the cell theory. Joining at once in the experimental attack, he secured results on *Amphioxus* (1892–93) that indicated an even greater freedom from the limitations of mosaic development than seen by Driesch in the sea urchin.

But Wilson reacted differently from Roux and Weismann, on the one hand, who proceeded to "explain" their findings by setting up an elaborate scheme of segregation of hereditary materials, and from Driesch, on the other hand, who finally drifted to the mystical assumption of an "entelechy" within the cell. He had before him the lessons not only of these experiments but also of the findings which he had so recently made in his comparative studies on the development of *Nereis* and other teloblastic animals and which indicated that, although these cleavages were in a sense very definitely determined, they might at the same time, in a more ultimate sense, be plastic. Keeping his balance in the face of these cross-currents, he arrived at the more modest and yet truer conclusion (1893, 1899) that the manner of development of a given part is "a manifestation of the general formative energy acting at a particular point under given conditions, . . . the formative processes in special parts [being] definitely correlated with the organization of the entire mass." He did not suppose that such words explained the nature of the "field" or of the "organizer materials," as they are now termed, but this should not obscure the fact that there is a definite and important meaning here. That is, that the nature of a given part is determined only through a process of interaction between that and other parts of the whole. Wilson and O. Hertwig independently came to much the same conclusion on this matter (except that Wilson came to regard the interaction as to a considerable degree potentially independent of the state of division of the whole into cells) and for a time Driesch too accepted this view. It is easy now to overlook this meaning, because of the lack of knowledge of the nature of the interactions in question, and of the invisible or-

ganization resulting from them. The need for such knowledge was, however, realized by Wilson, more perhaps than by the others of that time.

One of the directions of attack on these problems lay in experiments in which the nuclei were shifted or interchanged in their positions. In some early experimental work on sea urchins Driesch (1892) had shown that when the arrangement of nuclei in cleavage stages is changed by pressure the resulting embryos nevertheless give rise to normal larvae. This was, however, not surprising in a form in which the blastomeres so long remain relatively undifferentiated. Wilson first verified this finding and then (1896) tried the same experiment on *Nereis,* where differentiation of blastomeres had been so early evident. He found that even here normal larvae were produced, despite the fact that the nuclei had been so redistributed that many of them were contained in regions—and even in germ layers—other than those in which they normally would have lain. This greatly strengthened the conclusion concurred in by Wilson, Hertwig and Driesch that differentiation does not result from a segregation of nuclear material.

The ultimate equivalence of nuclei thus indicated remained, however, in striking contrast to the obvious segregation of developmental materials—now inferred to be cytoplasmic—which had been found in some of the experiments on separation of blastomeres. Thus, even in *Amphioxus* Wilson had found that the isolated blastomeres were far from uniform in showing immediate "total" development. And extreme mosaic types had now been found—for example, tunicates and the gasteropod *Ilyanassa,* both worked on by Crampton when a student of Wilson's—in which the development was much more regularly "partial" than in the amphibians that had been used by Roux to illustrate this principle. In what then lay the difference between these two types of development, termed by Conklin in 1898 the "indeterminate" and the "determinate" types? Could one say

that in the first there is an equivalence of blastomeres, together with "epigenetic" development, while in the second type development follows His's (1878) principle of "organbildende Keimbezirke" or Lankester's version, "precocious segregation"? Wilson considered it very unlikely that there should be a fundamental difference in the principles governing the development of the two seemingly so different types. He succeeded very well in reconciling the apparent discrepancies by referring them to variations in the time at which the underlying differentiations took place, in relation to the cleavage divisions.

Putting together all the facts available, Wilson concluded that development in all cases is fundamentally an "epigenetic" process, in that if the egg be traced back to an early enough stage of its formation—often, to an early stage within the ovary—its cytoplasm is found to be relatively undifferentiated regionally and even its polarity is dependent upon its relations with its neighbor cells. (Sometimes—as Wilson found to be the case in the echinoderm egg—this polarity is not the final polarity, which is dependent on the chance point of entrance of the spermatozoon.) In time, however, the egg must become transformed from this relatively amorphous condition into a cell or (depending on the timing of the process in relation to cleavage) group of cells in which the different "organ-forming materials" are to a greater or lesser degree sequestrated into different regions. By the time this happens, then, there is truth in His's contention that some kind of topographical organization exists which, though it may yet be invisible, helps to determine the later visible differences. Now if the broad features of this organization have already been laid down with considerable finality before cleavage commences—and if (as was later added) they are so arranged that parts of it will become separated by the early cleavage planes—the egg shows the type of development classed as "mosaic" or "determinate." Otherwise it will be classed as "indeterminate." In most cases, however,

some at least of the processes of differentiation in a sense never reach completion, even in the adult, but only approach an equilibrium condition that can still be upset at any time, as by cutting, with resultant regeneration, which after all represents only "a survival, in the adult, of a condition characteristic of the embryonic stages" (Wilson, 1898). In the embryo, the process of cell division often helps to demark the invisibly or visibly differentiated regions, yet the cells as separate units play only a secondary role in differentiation. As for the nuclei, those of the different cells, in the early stages of development at least, are to be regarded as equivalent in potency to one another and to the nucleus of the fertilized egg, even though each nucleus, within itself, must be highly organized and though the processes of differentiation themselves are ultimately referable to the activities of these nuclear materials.

It may here be pointed out that although Wilson's experiments along the above lines were mostly conducted upon eggs and embryos, they were not always confined to these stages. For example, Wilson in 1902 studied the mode of interaction of the parts by regeneration experiments on the adult stage of the polyp, *Renilla,* whose development he knew so well. He showed that in this material (as was by that time known for planarians) a direct transformation of given parts into others takes place, which is in this case dependent to some degree upon mechanical factors. In other experiments, transformation of germ layers (in the sense of parts normally formed by one germ layer now being formed by another) was noted by Wilson. All this emphasized the importance of the mutual interactions in determination, together with equivalence of nuclei.

The very fact that the above set of principles are today so taken for granted by biologists makes it the more difficult for them to put themselves back to the time when the road to their attainment was so beset with obstacles. Moreover, as different points had been originated by dif-

ferent investigators, many of them contending with one another and advocating elaborate schemes of their own, it was a task requiring especial objectivity, judgment and insight so to cull out, fit together and integrate the elements of value contained in the various erring, warring doctrines as to arrive at that synthetic point of view of development which we now have.

In 1902 at the South Harpswell Laboratory, Maine, and in 1903 during Wilson's third stay at Naples, he carried out an extended and striking series of experiments on the development of types with spiral cleavage: the Nemertean worm *Cerebratulus* (in Maine) and the mollusks *Patella* and *Dentalium* (in Naples). This work led mainly to a strengthening and extension of his previous conclusions. If cut in any direction shortly after maturation, before fertilization, the Nemertean egg proved to be equipotential in regard to the cytoplasmic determiners for all the distinctive features seen during the cleavage and larval (pilidium) stages. But if cut after fertilization it had become mosaic (*i.e.*, "organized" or "prelocalized"), as shown by the partial blastulae (nevertheless capable of considerable later regeneration) formed from such fragments as well as from isolated blastomeres. The development of pieces cut still later—*i.e.*, in the blastula stage—showed that by this time still further "prelocalization" had set in. Yatsu, carrying this work further, under Wilson's guidance, showed the gradualness of onset of these localizing processes in the *Cerebratulus* egg, their orientation along certain planes and the similarity in time and manner of prelocalization of the factors affecting later stages to those affecting the stages studied by Wilson.

In *Dentalium* and *Patella*, unlike *Cerebratulus*, Wilson found that the localization was already well advanced considerably before fertilization. A certain region in the lower half of the unfertilized egg had already come to contain materials necessary for the formation of a whole series of important parts of the larva. When this

region is cut away either in the unsegmented egg, when it is still unrecognizable, or in cleavage stages, when it periodically appears as a large "polar lobe," these parts do not develop at all. An exception to this rule is found in the apical organ. This is not formed in the region of the embryo corresponding to that part of the blastomere stage where the polar lobe was located, yet its formation is dependent upon the presence of the latter through the time of the two-cell stage; if, however, the polar lobe is not removed until after this stage the apical organ will be able to develop. The localization of the polar lobe material in the egg stages is only the earliest example of such processes here. As development progresses one premitotic localization follows another in the most elaborate fashion so that in fact each cell up to the 64-cell stage continues to form practically the same parts, if separated from the rest, as it would have done if left in its natural position in the whole. It thus becomes evident that localization of the materials necessary for the development of ever more circumscribed parts continues to occur. That is, "the divisions that would be considered as qualitative or quantitative from the point of view of descriptive cell-lineage, are really such as regards the inherent factors of differentiation. The descriptive and comparative study of cell-lineage represents something more, therefore, than a mere enumeration of successive cell divisions and their geometrical relations, and has the value of a direct examination of the normal morphogenic process" (Wilson, 1904).

At the same time, the shapes, positions and sizes of the cells formed even by these extreme mosaic embryos were shown not to be entirely free from regulation by other parts, and this was, as expected, more evident in the earlier stages. For instance, an egg fragment or a $\frac{1}{2}$ blastomere containing the polar-lobe material could form virtually a whole larva, and even the size of the polar lobe and its derivatives formed by a fragment having originally a great excess of polar lobe material tended to be

normal in relation to the size of the other parts present. And the influence of the polar lobe on the formation of the apical organ seems to furnish another illustration of regulative action, as Wilson later (1929) pointed out.

A quarter century later, at the age of 72, Wilson resumed his experimental attack on these problems, studying the cleavage of the egg of the annelid *Chaetopterus* after its fragmentation by centrifuging. If the eggs were fragmented prior to a critical period that occurred shortly after their maturation, the majority of the fragments, on being fertilized, underwent normal cleavage (and some formed whole larvae) even though the visible materials had been abnormally distributed by the centrifuging and many fragments had received only the relatively clear "ground substance." Thus the conclusion could be drawn that it is in this clearer portion of the cytoplasm that the processes of pre-localization and differentiation normal to the embryo occur.

Once more reviewing the problem of localization versus regulation, it was shown in his last papers on the subject (1929, 1930) that the evidence had become stronger than ever both of pre-localization in the more markedly regulatory eggs and of regulation in those of more conspicuously mosaic type. It was also pointed out that, in fact, the two processes can not be considered as fundamentally distinct, both alike involving the production, more or less limited in time and in spatial distribution, of what were once termed "formative substances." The newer results on "organizers" likewise were shown to be of a piece with this general scheme; a probable illustration of this relationship was furnished by the manner of determination of the apical organ of *Dentalium*, above mentioned. The recognition of the superficiality of the distinctions here involved were, however, combined with a realization that this should only help in clearing the ground for the real work of experimental analysis of development, and especially of the peculiar "formative" processes that cause localizations of materials within definite regions of cells

or cell-aggregates to occur or not to occur, in accordance with whether or not certain other regions are present. Such work (as he had stated in 1914, then with reference to biological progress in general) would require that the investigator "be thoroughly trained in the methods and results of chemistry and physics."

In line with the above point of view and in opposition to Driesch and his sympathizers, Wilson on various occasions was led to reiterate (though usually in unprovocative language) his position that "*the scientific method is the mechanistic method*" (1903—italics his). It should be unnecessary to explain that this term was not used by him in the sense of crude mechanics, but in the same sense as usually employed by English-speaking scientific writers, namely, as the antithesis of the term "vitalistic" and so as practically equivalent to "materialistic." Wilson was, however, too clear-sighted a scientist to be drawn into idle controversies over such terms.

That a "mechanistic" analysis (in the above sense) of fundamental cellular phenomena still had much to learn through a combination of exact observation and experiment on things of microscopic size, before these could receive their explanation entirely in the terms of the chemists and physicists, was, however, much clearer to Wilson than to those whose voices were beginning to be heard in favor of "*experiment alone*" (see Wilson's criticism of them in 1900), and some of whom refused to consider even an experiment scientific unless it made use of the technicalities of chemical or physical formulae. The short-sightedness and extreme oversimplification characterizing this position was in fact proved by nothing better than by Wilson's own work. In this connection we rightly think in the first place of the major work of his later years —that on chromosomes—but he was led to this gradually, from 1891 to 1901, as it became possible for efforts applied in this field to be more productive of results. In the meantime—and again in his later years—Wilson's main researches were along two lines correlative with this: (1)

the studies, above outlined, of the invisible organization of the cell in relation to the processes of early development, and (2) studies—also carried out mainly on eggs —of the properties of the visible achromatic constituents common to cells in general.

Inquiries into the Material Basis of Mitosis

Examining the minutiae of cytoplasmic structure through their gradual transformations in the growth of the echinoderm and other eggs, Wilson found in 1899 that, although the cytoplasm finally becomes alveolar and thus comes to agree with Bütschli's conception of its universal conformation, this condition is attained by enlargement of extremely fine, scattered granules. A further substantiation was thereby provided of the view, for which some investigators were already contending, that neither an alveolar configuration nor a microscopically fibrillar or reticular one is fundamental or permanent.

In this and earlier papers (1895) on echinoderm egg material Wilson also paid particular attention to the achromatic structures connected with mitosis. It was demonstrated by him, partly in collaboration with A. P. Mathews, then his student, that Fol's "quadrille of centers" story of the origin of the centrosomes and asters at fertilization was wrong. Instead, it was shown, both asters of the first cleavage stage are derived from the sperm aster by division of the latter, and they in turn give rise, by their repeated division, to all the asters of later stages, as pictured in Boveri's scheme of the fertilization process. This material was, however, not well adapted for the observation of the central bodies within the asters. The evidence of various workers on other material seemed to show clearly the genetic continuity of these granules, yet the question remained in dispute. Long afterwards—in 1930 and 1931—Wilson returned to this question and—partly in collaboration with Huettner, who had been his student—gave clear-cut evidence of the persistence and reduplication of the central bodies

throughout the normal embryonic development of *Drosophila* and other forms.

As for the nature of the astral rays and spindle fibers, Wilson inferred from his observations of the 90's that they exist as actual fibers. Later, however (1901 *et seq.*), on the basis of newer observations and experiments showing the rapidity of the aster's movement and of its disappearance and reappearance with anesthetization (see below), he came to regard the rays at least as coagulation products and to favor a view of their representatives in the living cell as consisting of lines of flow or attempted flow, or at any rate of orientation, somewhat as had been proposed by Bütschli. Even in the earlier papers, however, he had not adopted the oversimplified fibrillar scheme of the mechanism of mitosis that had been advocated by Van Beneden and Heidenhain. For he argued that a mere fibrillar contractility of the spindle fibers could hardly cause the movement of the chromosomes to continue all the way to the poles, as he had observed it to do in the echinoderm eggs. He was therefore disposed to regard the movement as caused not by ordinary contraction of fibrils but by traction of some other kind.

In the late 80's and the 90's a series of novel observations and experiments on imperfectly fertilized and unfertilized eggs—by the Hertwigs, Ziegler, Mead, Morgan and others—had shown that eggs of species in which fertilization normally occurs are on occasion able to undergo maturation and/or some degree of cleavage without a sperm nucleus in the dividing cell and even without a spermatozoon at all. This presently culminated in Loeb's startling discovery of "artificial parthenogenesis," *i.e.*, that normal development can be artificially induced without a spermatozoon. Immediately thereafter, Wilson took up Loeb's method and, making the first accurate cytological study of the phenomena of artificial parthenogenesis, was able (1901) to throw new light on the nature of the mitotic structures and on the mechanism of mitosis. It had been thought that the centrosomes of

Wilson at thirty-three, in the heyday of his exploration of cell lineage. This was in 1890, his fifth year at Bryn Mawr, and his last before accepting the call to Columbia.

the cleavage stages were in this case formed from the achromatic portion of the egg nucleus, but Wilson (using the sea urchin *Toxopneustes*) showed that they arise in the cytoplasm (*de novo*), and he produced them even in egg fragments lacking a nucleus. Those that later became connected with a nucleus could act as normal centrosomes in cleavage, but artificial asters lying in "cells" lacking a nucleus (like the asters derived from sperm but separated from their nuclei in Boveri's earlier cases of "partial fertilization") also went through the motions of mitosis periodically. Without a nucleus, however, they seldom acted powerfully enough to effect complete cleavage of the cytoplasm.

When, as sometimes happens, centrosomes connected with a nucleus failed to divide, monasters were formed. Such figures had occasionally been seen in earlier work on fertilization, and it had been noted that they underwent some sort of pseudo-mitosis, but it was now shown by Wilson that in such cases not only do the chromosomes periodically condense but they undergo regular division with subsequent separation of the chromatids along the lines of the spindle fibers as in the early anaphase of regular mitosis. Thus the single reconstructed nucleus comes to have an ever doubling chromosome number.

It was evident from the above observations, taken together, that centrosome doubling and regular chromosome doubling do not necessarily depend, either one, upon the other. That there is, however, an underlying cytoplasmic rhythm in mitosis, to which the rhythm of both the centrosomes and the chromosomes may be secondary, was shown by Wilson in his work of a few years later on *Dentalium*, where he observed rhythmic changes in fragments of fertilized eggs lacking both centrosomes and nucleus, occurring synchronously with the mitoses in the complementary nucleated fragments.

Additional light on mitosis was shed by other experiments of Wilson's in the same year. In these he studied the effects of obliterating the astral figures in the fer-

tilized eggs by etherization—a phenomenon that had been discovered and to some event studied by the Hertwigs in the case of other anesthetics—and of obliterating the cleavage furrows by shaking—a method discovered by him. Both the movement of the pronuclei towards one another from a distance and the cleavage of the cytoplasm proved to be processes dependent upon the presence of well-formed asters, while separation of the two daughter chromosome-groups from each other was more or less complete according to the degree of development of the spindle. With moderate etherization or with shaking, division of the nuclei could occur without that of the cytoplasm. In cases of the latter sort, after recovery from the ether or from the shaking, not only might cleavage occur at the next mitosis along the planes cutting across the existing karyokinetic spindles, but also along the planes where all the previous cleavages (even remotely past ones) should have been—provided the asters still lay near enough to these planes. In the discussion, Wilson used the above and other results as further evidence for the exertion of a tractive force by the centers, similar in principle to that in Bütschli's hypothesis.

Preparing Cytology for Mendelism

Although Wilson had not yet, before 1900, carried out any major investigations dealing primarily with the "chromatin" (using this word here to denote the substance of the chromosomes, in general), he was not only very much aware of its importance, but had played an active part—among English-speaking scientists by far the most active part—in bringing this realization home to others. His own researches had had the effect of confronting him with cumulative evidence of the preponderant role of the nucleus in the ulterior processes of determination, and so had his careful studies of the literature. All this was, however, but a part of the organized view of cellular phenomena in their relation to the main problems of development, heredity, evolution and life

processes in general that he had now arrived at. Owing to his objectivity, his historical perspective and his faculty of extracting and integrating with each other the better elements of opposing ideas, this general conception was much in advance of anything that had previously been expressed in English and has in fact met the test of later work better than any other equally comprehensive synthesis of this field made at that time. Wilson's papers on individual problems could not, however, adequately convey his rounded conception, despite their exceptionally high value for historical orientation with reference to the given topics. It is therefore fortunate that, in his first decade at Columbia—1891 to 1900—Wilson spent considerable time in gathering together all this material and presenting it in the form of a monumental book, "The Cell in Development and Inheritance" (first edition, 1896; second, 1900). The basis for this book had been laid in a course of lectures given in 1892–93.

The book was dedicated to his dear friend Theodor Boveri, whom he had known at Munich and Naples and whose brilliant experiments and ideas on chromosomes had influenced Wilson more strongly than had the work of any other biologist of Wilson's own generation. "The primary intention of the work," as he stated in the introduction, was to contribute to the attempt (the keystone of which, according to Wilson, had been supplied by Weismann's arguments concerning the continuity of the germ plasm) "to bring the cell-theory and the evolution-theory into organic connection." This does not mean that it was intended to enter into any discussion of evolution itself, but to present the whole status of the cell-theory in such a way as to make it useful material for the further understanding of evolution and of those fundamental processes —such as heredity, variation and the determination of characters—on which it was becoming clear that evolution must be based.

How well Wilson succeeded in this purpose—considering the state of knowledge of that time—could not have

been nearly so evident then as to those reading the book of 1896 or 1900 to-day, in the light of the now established general theory of genetics. Not only would it be a good thing for all beginners who expect to devote themselves to genetics or related fields to read this book in an early edition, but those already engaged in such lines who have not read it for many years will find themselves well repaid by a rereading of it now. Many of them will be amazed at the foreshadowing of our modern conceptions therein presented, and at how well the stage for genetics had already been set. It will be forcibly brought home to them what an accumulation of labor on the part of a multitude of patient inquirers, and how many flashes of insight—right and wrong—have been involved in the establishment of our simple-seeming general theories, how long a valid inference which is too good (too far ahead of its time, as the saying is) must sometimes wait for recognition, how very near a host of workers may come to the understanding of the essentials of some important phenomenon (*e.g.*, segregation, in pre-Mendelian days) while still missing the key point (in this case, pairing of likes) for the lack of some simple element (differences between chromosomes of the same set) that apparently might have been supplied by a very small step of reasoning, experiment or observation, how wrong and right at the same time many a hypothesis may be (*e.g.*, Weismann's idea of reduction or of the composition of chromosomes or His's idea of the organization of the egg), and how valuable is the work of one like Wilson who, in bringing the various facts and theories into due relation with one another, is able to point out where solid ground lies and to indicate in what direction further efforts might usefully be exerted. Moreover, from a reading of this book to-day, the reader is likely to emerge with an invigorated confidence in the progress of science and an increased respect, if not for men in particular, then for man in general—a feeling that to-day may be sadly faltering. And this respect will know no boundaries of nations.

"The Cell" gives a comprehensive treatment of the visible features of cell structure, mitosis, the formation of the germ cells, fertilization and the earlier stages of development, all in terms of the researches through which the facts were found, and with the aim constantly in view of shedding light on the nature of the basic life processes, on the mechanisms of development and of heredity, and so too, ultimately, on the process of evolution. The marshalling of the myriad facts leads Wilson, through a series of converging channels, to become increasingly emphatic, throughout the course of the book, in support of the conclusion—which, as he says, had been reached in 1884–85 by "Hertwig, Strasburger, Kölliker and Weismann independently and almost simultaneously"—"*that the nucleus contains the physical basis of inheritance; and that chromatin, its essential constituent, is the idioplasm postulated in Nägeli's theory*" (italics his). This is in fact rightly to be regarded as the central conclusion of the book (though in places Wilson still, with his characteristic caution, terms it "a working hypothesis")—much of the rest forming evidence in some way contributory to it or having a bearing on the manner in which the principle works out.

The chief lines of evidence for this conclusion then recognized by Wilson were the following (the same arguments being found in both the first hand and second editions):

(1) The surpassing accuracy with which the chromosomes, by longitudinal division, *i.e., in all their parts,* are distributed at mitosis, in contrast with the cytoplasm, as pointed out by Roux in 1883 in arguing for the linear differentiation of the chromosome materials. This argument and certain supporting observations on the chromomeres and their division "point," said Wilson, "unmistakably to the conclusion that these granules are perhaps to be regarded as independent morphological elements of lower grade than the chromosomes." However, he agrees with Strasburger that the microscopically

visible bodies may not be the "ultimate dividing units." Of the latter he remarks: "Somewhere, however, the series [of smaller and smaller constituent parts of the chromosomes] must end in final chromatic units which cannot be further subdivided without the decomposition of chromatin into simpler chemical substances; and these units must be capable of assimilation, growth, and division without loss of their specific character."

(2) The indications, set forth by Rabl (1885) and Boveri (1887 *et seq.*), that the chromosomes, despite their seeming disappearance as such during the resting stages, retain some sort of individuality. The evidence of that time lay in the tendency of the chromosomes to reappear in their old positions after each resting stage in the series of the embryonic cell cycles, and in observations, made subsequently to this by Boveri, that at each reappearance they are of the same number and morphological types as they were to begin with, even when the original group of chromosomes had in one way or another been rendered an abnormal one. In 1895 Wilson had still been skeptical of the chromosomes' retaining their individuality, but by 1896, after Boveri's conclusions had received further support from findings of Häcker, Rückert and others of the separateness of the maternally and paternally derived chromosomes in the early stages of *Cyclops* and *Ascaris*, Wilson became a strong advocate of this doctrine. He was not yet convinced, however, that this individuality consisted in a retention of the actual identity of the chromosome material, and hence preferred to speak of the "genetic continuity" rather than the "individuality" of the chromosomes.

(3) "The whole process of maturation, in its broadest sense, renders the cytoplasm of the germ-cells as unlike, the nuclei as like, as possible. The latter undergo a series of complicated changes which result in a perfect equivalence between them at the time of their union, and, more remotely, a perfect equality of distribution to the embryonic cells. The cytoplasm, on the other hand, un-

dergoes a special differentiation in each to effect a secondary division of labor between the germ-cells. When this is correlated with the fact that the germ-cells, on the whole, have an equal effect on the specific character of the embryo, we are again forced to the conclusion that this effect must primarily be sought in the nucleus, and that the cytoplasm is in a sense only its agent."

(4) The experiments of Nussbaum, Gruber, Verworn and others, in which cells of various kinds—protozoa, cells of plant hairs, etc.—were separated into nucleated and enucleated parts by cutting, plasmolysis and other means. These indicated that the presence of a nucleus was necessary for processes of constructive metabolism: growth, regeneration and apparently even assimilation. Moreover, observations on the position assumed by the nucleus in cells of different types and in different stages of their functioning indicated that the nuclei tend to lie in proximity to the regions of cells where active constructive processes are long continued.

As to the explanation of how the chromatin is given this superiority over the cytoplasm in development and heredity, Wilson realized that the problem was ultimately a chemical one. He discussed the chemistry of the chromatin and other constituents of the cell, as then known, in some detail, but made it evident that such work was not yet sufficiently advanced to disclose the mechanism involved. At that time, however, he leaned toward the hypothesis of Kossel, as carried further by Mathews (who, as Wilson's student, had in turn been influenced by him) "that nuclein . . . may in a chemical sense be regarded as the formative center of the cell which is directly involved in the process by which food-matters are built up into the cell-substance" and in "the synthesis of complex organic matters." And this, as Wilson says, is "in harmony with the hypothesis advanced twenty years ago by Claude Bernard (1878), who maintained that the cytoplasm is the seat of destructive metabolism, the nucleus the organ of constructive metabolism and organic syn-

thesis, and insisted that the role of the nucleus in nutrition gives the key to its significance as the organ of development, regeneration, and inheritance." Expanding upon this, Wilson says (in both editions alike):

> In its physiological aspect, therefore, inheritance is the recurrence, in successive generations, of like forms of metabolism; and this is effected through the transmission from generation to generation of a specific substance or idioplasm which we have seen reason to identify with chromatin. . . . If the nucleus be the formative centre of the cell, if nutritive substances be elaborated by or under the influence of the nucleus while they are built into the living fabric, then the specific character of the cytoplasm is determined by that of the nucleus, and the contradiction [that a specifically organized cytoplasm is necessary for the nuclear activity] vanishes. . . . The nucleus can not operate without a cytoplasmic field [*sic*!] in which its peculiar powers may come into play; but this field is created and moulded by itself.

The cellular biology of 1895 to 1900 was furthest behind that of to-day in its knowledge of meiosis. An important part of the clue to this great series of phenomena had, as Wilson recognized, been given by Weismann in his remarkable deduction of the existence of a reduction division, effecting a qualitative separation of hereditary (chromosomal) constituents. Unfortunately, Weismann had depicted this as involving either transverse division or separation of chromosomes. The observations seemed in part to tally with the idea of some sort of qualitative reduction, but the various accounts of the complicated conformations of meiosis were extraordinarily difficult to interpret and to reconcile with one another, since it was assumed by those who already believed in a binary conjugation of the chromosomes that the juxtaposition was endwise. For neither the conception of parasynapsis (Winiwarter, 1900) nor that of homologous chromosomes (see below) had yet been launched. The side-by-side conjugation plainly seen in some objects was thus mistaken for longitudinal division, and the resultant appearance of double longitudinal division at meiosis in these cases seemed to leave no room for reduction at all. On the other hand, Weismann's idea of transverse chromosomal division at reduction, which was widely interpreted

as separation between chromosomes that had been joined end to end, was supposed to have been proved in some cases, and as an explanation of reduction it fitted in well (without the necessity of postulating homologous chromosomes) with his over-elaborated idea of chromosome structure. According to this, each of the numerous chromomeres or "ids," in every chromosome of a germ cell, contains a complete set of determiners for the individual. On this conception the chromosomes must all be nearly equivalent and conjugation need not be precisely ordered.

Perhaps it was the diversion of attention caused by this idea that so long retarded the discovery of parasynapsis and of the simple fact that the different chromosomes of a given individual are often recognizably different from one another. The latter observation, combined with the already extant doctrines of equivalence of male and female germ nuclei and persistence of chromosome individuality, must rapidly have led to the conception of homologous chromosomes and then to its corollaries, synapsis and segregation of the homologues. The existence of pairs of homologous maternal and paternal chromosomes was in fact finally proposed by Montgomery, in 1901, apparently independently of Mendelism. Had this happened a year sooner, "Mendelism" would have been independently arrived at by cytologists, and the confusing cytology of meiosis might sooner have been disentangled.

But despite these pre-Mendelian difficulties, some of which, like the claim that there is a double longitudinal division in meiosis, greatly disturbed Wilson, he declared that:

> The peculiarities of the maturation-mitoses are obviously correlated in some way with the numerical reduction, and the fact that they differ in so many ways from the characters of ordinary mitosis gives ground to hope that their exhaustive study will throw further light not only on the reduction problem itself but also on mitosis in general and on still wider problems relating to the individuality of the chromosomes and the morphological organization of the nucleus.

In accordance with this view concerning the direction of the trail ahead, Wilson himself, with some of his students—*e.g.,* Calkins and McGregor—were already actively at work upon the cytology of the maturation stages, in a variety of forms.

On the last page of both early editions, Wilson called attention to the complete lack of knowledge of the time concerning the mechanism of variation and to the fact that "study of the cell has on the whole seemed to widen rather than to narrow the enormous gap that separates even the lowest forms of life from the inorganic world." He expressed his "conviction that the magnitude of the problem of development, whether ontogenetic or phylogenetic, has been underestimated." Nevertheless, he concluded, "the splendid achievements of cell-research in the past twenty years stand as the promise of its possibilities for the future, and we need set no limit to its advance. To Schleiden and Schwann the present standpoint of the cell-theory might well have seemed unattainable. We cannot foretell its future triumphs, nor can we doubt that the way has already been opened to a better understanding of inheritance and development."

(*To be concluded in the March–April issue*)

ADAPTATIONS IN THE NASAL PASSAGES FOR SAND BURROWING IN THE SAURIAN GENUS *UMA*[1, 2]

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MOSAUER (1932) in his study of adaptive convergence in the sand reptiles of the Sahara and of California gave considerable attention to certain of the sand adaptations in *Uma notata*, the Ocellated Sand Lizard. However, the nasal structure of this lizard has been given only a cursory examination. The writer's investigation is directed toward a more complete study of the olfactory apparatus both as to structure and functioning in an effort to explain successful subsurface respiration in this genus.

The lizards of the iguanid genus *Uma* are confined to deserts of the southwestern United States and Sonora, Mexico. They are characterized by elongate, pointed scales on the sides of the digits and an ocellated dorsal pattern.

These reptiles inhabit aeolian sand, characteristic of arid regions (see Fig. 1), and have developed the habit of submerging beneath the surface of this material for protection. Life in the sand has left its mark in numerous adaptations, one of which has given rise to the vernacular name "Fringe-foot."

These lizards spend much of their time beneath the ground. Since the animals are surrounded by minute, freely movable particles which are smaller in size than the external nares, a problem of respiration beneath such material is encountered. These facts led to the examination of the nasal apparatus. The existence of a device whereby small particles are filtered out of the inspired air was suspected.

[1] Condensation of a thesis submitted in partial fulfilment of the requirements for the degree of master of arts. Work carried on under the direction of Professor R. B. Cowles, of the Zoology Department, University of California, Los Angeles.

[2] Illustrations by the author.

The following account describes first the gross and microscopic structure of the nasal passages and adjacent features and secondly the function of these channels in one aspect of the ecology of these reptiles.

Following the review of the genus *Uma* made by Heifetz (1941) *Uma notata notata* and *U. inornata* have been used most extensively in this study. No appreciable difference between these forms has been detected in

Photo by R. B. Cowles

FIG. 1. Hummocks of wind-blown sand resulting from the deposition of particles impeded by scattered vegetation. *Uma inornata* frequents this deposit located a few miles northwest of Thousand Palms in the Coachella Valley.

the morphology and function of the nasal passages. *Uma scoparia* has been given a cursory examination. This species is similar to the other two and therefore the statements made here are equally applicable to all the known members of the genus.

ADAPTATIONS IN THE NASAL STRUCTURE

Uma has successfully coped with the problem of respiration beneath a medium which in its mechanical proper-

ties has a general resemblance to those of a fluid. The largest particles of sand are much smaller than the nostrils of the lizard and the smallest fragments approach in minuteness particles which can be called dust. Respiration is carried on underground for hours at a time without any apparent inconvenience to the reptile. The nasal mechanism and its functioning is such that sand is prevented from reaching the lungs. Thus these vital organs are adequately protected from foreign particles.

GROSS MORPHOLOGY

In *Uma* the nasal passages are tubular, forming U-shaped channels. The nostrils are situated dorsally and anteriorly on the snout. They are elliptical in shape with their long axes at an angle to the main axis of the head, the anterior edges diverging.

To facilitate description, the path of only one of these paired structures will be followed. Attention to the illustrations (Plates I and II) is essential for an understanding of this rather complicated breathing tube.

The external naris opens into a passage, the diameter of which is about the same as that of the nostril. This passage extends medio-ventrally, turning abruptly and continuing posteriorly just beneath the bones of the nasal region to a point near the eyeball. The passage doubles back on itself and proceeds anteriorly with a constant diameter approximating that of the dorsal limb of the tube, terminating in a depressed, oval chamber, the principal nasal cavity. On the antero-lateral portion of the floor of this chamber is located the naso-pharyngeal canal which extends at an angle, medio-ventrally into the mouth by way of the internal naris (Plate II, Fig. 2).

A conspicuous feature of the gross morphology of the nasal region is a large gland, the glandula nasalis lateralis, which rests upon the dorsal wall of the lower limb of the nasal tube adjacent to the dorsal limb (Plate I, Fig. 1). This gland opens by two ducts at the extreme posterior portion of the lower limb.

Microscopic Structure

Nasal Valve: The region of the vestibule adjacent to the external naris is highly vascularized. Blood and lymph flowing into the tissue of the floor of the vestibule proximal to the external nasal aperture cause a swelling which may almost completely block the opening. This valve-like floor possesses an abundance of smooth muscle fibers which underlie a stratified squamous epithelium. The region is free of glandular elements.

Epithelium of the Nasal Passages: Vestibule. The lining of the dorsal limb of the nasal passage is a stratified squamous epithelium which is uniform in thickness throughout. It is devoid of glands. This layer lines the cavity of the vestibule which doubles on itself posteriorly, extending to the anteriorly situated oval chamber, the principal nasal cavity (Plate I, Fig. 1). This cavity, along with a small segment of the vestibule, together comprise the ventral limb.

Principal Cavity: The oval chamber itself is lined throughout with a ciliated, pseudostratified epithelium composed of two types of cells, those which comprise Bowman's glands, the multicellular and unicellular mucus secreting structures and likewise the non-glandular, ciliated elements. The cilia uniformly cover the entire inner surface of the principal cavity (Plate II, Fig. 1). These cilia are extremely abundant and are short and thick, almost bristle-like in appearance.

Cilia extend posteriorly along the roof of the mouth. This ciliated epithelium is largely confined to grooves which extend posteriorly from the internal nares. These grooves converge medially to form a single channel which passes toward the throat (Plate I, Fig. 2, and Plate II, Fig. 1). No cilia are present anterior to the choanae.

Glandula nasalis lateralis: The tissue of the lateral nasal gland is comparable in structure to mucous gland tissue in mammals. The gland is composed of numerous tubules lined with cuboidal epithelium. These tubules lead to larger ducts which are lined with columnar epi-

thelium. These larger channels finally unite to form two master ducts which pour the secreted products into the nasal tube just above the principal nasal cavity (Plate II, Fig. 1). The tubules and ducts are held together in a matrix of loose, parenchyma tissue.

Walls of the Nasal Passages: The walls of the nasal passages of *Uma* are highly vascularized. Numerous vessels form a network surrounding the nasal tubes. Smooth muscle fibers, arranged for the most part with their long axes at right angles to the nasal epithelium, are in a position to dilate the nasal tubes by their contraction. These fibers are attached to the nasal epithelium on the one hand and to the adjoining connective tissue system, cartilage, fibrous connective tissue or bone as the case may be on the other. An influx of blood into the vessels about the tubes would cause a narrowing of the passages. The nature of the vestibular tissue is not unlike erectile tissue as found in certain mammals. A mechanism is thus present for the control of the diameter of the nasal tubes.[2]

FUNCTION OF THE NASAL APPARATUS IN UMA

Sand grains are irregular in shape and size and when dry are freely movable over one another. Under these conditions air spaces occur continuously throughout the fragments. There is thus an abundance of air in the interstices between the sand particles which compose the substratum of the habitat of *Uma*. The problem is to obtain this air during subsurface respiration without at the same time drawing particles of sand into the vital respiratory structures.

[2] Bruner (1907) describes a sinus vestibuli nasi which occurs in the region of the external naris in certain reptiles. This vascularization of the tissues of this region comprises a portion of a general system of cephalic venous sinuses present in reptiles. Briefly it may be stated that these sinuses can be dilated by contraction of a ring of striated muscle fibers about each of the internal jugular veins which causes a damming up of the blood in the head. The vascularized walls of the nasal passages in *Uma* constitute a portion of this system of cephalic veins. The same stimulus that initiates an influx of blood into the sinus vestibuli nasi resulting in an elevation of the floor of the nasal passage adjacent to the external naris (page 41), doubtless concomitantly causes a reduction in the diameter of the nasal tubes.

When *Uma* lies buried near the surface and is respiring rather vigorously, funnel-shaped pits form in the sand over each nostril (see Fig. 2). Examination of the nostrils, which appear occasionally at the bottom of the pits during expiration, reveals that the apertures have been narrowed considerably by elevation of the floor of the nasal tubes. However, it has been observed that particles move freely back and forth through the crescentic

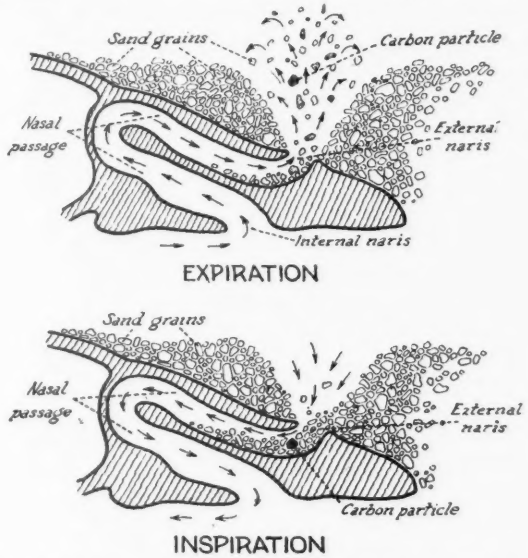

FIG. 2. Semi-diagrammatic representation of a sagittal section through one of the nasal passages of *Uma* showing the movement of sand particles during respiration at a superficial level in the soil.

openings regardless of the valvular action. The function of the valves minimizes but does not completely stop the entry of grains.

With each expiration, which is quite explosive in character, the sand is blown into a jet of particles which rise and then fall back about the periphery of the funnel as well as into the bottom of the pit.

Upon inspiration the particles begin to move inward but soon interfere with one another, massing together

with the result that only an occasional two, three, or a half dozen grains disappear from view. Nevertheless, even if only a few particles were sucked into the nasal apparatus at each inspiration, in the course of a few hours an appreciable accumulation of sand would occur.

With this possibility in mind the fate of an individual grain was determined by carefully removing sand from the region of the head of a specimen which had just buried itself. This was done so as to leave only a thin layer of material over the external nares. Pits due to respiration were immediately formed over each naris. Within each pit the sand oscillated up and down with respiration. A particle of carbon, about the size of a sand grain, was placed in the pit. This fragment could be easily distinguished as it oscillated in a vertical plane with the other particles. Occasionally it disappeared into the nasal aperture but it invariably reappeared. This oscillation of the carbon particle continued for 20 minutes (see Fig. 2).

A repetition of the above experiment was performed with similar observations. This time a carbon particle was placed in each pit formed by respiratory action. After a few minutes of examination, with the results confirming the previous experiment, the pits were carefully covered with sufficient sand so that oscillation of particles was prevented. After several minutes the sand was removed revealing the carbon fragments at the external nares in a position corresponding to that observed in the open pits. It was thought that the added weight of overlying sand might cause inhalation of those sand particles near the nasal aperture.

The carbon particle experiment indicates that little sand reaches the lower limb of the nasal tube. A number of factors are involved: (1) A purely physical phenomenon, separate from any adaptive features of the nasal structure, is of considerable importance in the explanation of the successful subsurface respiration of *Uma*. There is a tendency for the angular sand fragments to

interfere with each other as they are drawn toward the nasal aperture with the resultant formation of a grill of interlocking particles which tends to prevent the inspiration of other fragments. (2) Ordinarily when *Uma* lies buried beneath the sand, the nasal tube is at an angle to the horizontal (see Fig. 2). Sand grains which reach the vestibule must be drawn up an inclined plane in order to reach the lower portion of the channel. They must be moved against the pull of gravity. The vestibule is without glandular elements, hence it is essentially dry, nevertheless its walls frequently exhibit a few adherent particles. However, most of the particles drawn in with inspiration are loose and for the most part are freely blown out with expiration. (3) The grains which adhere to the walls of the nasal tube in proximity to the external naris and about the aperture serve to impede the inward progress of free grains. (4) The valve at the external naris functions to reduce the amount of sand gaining access to the passages but does not completely prevent its entrance. This capacity was demonstrated by holding a living lizard with the nostril on one side of the head directed upward. A small amount of sand was poured into the opening. The result was an elevation of the floor of the passage, reducing the nostril to a narrow, crescent-shaped slit (Plate II, Fig. 1). The arrangement of the smooth muscle fibers in the nasal valve of *Uma* is such that with contraction there is a depression of the epithelial surface in this region. The elevation of the floor of the nasal passage results from an influx of blood and lymph into the sinuses of the valvular tissue in a manner suggested previously in connection with swelling of the lining of the nasal tubes. Bruner (1907) believes that the cephalic damming up of the blood dilates certain sinuses in the orbital region and elsewhere, facilitating exuviation by stretching and ultimately tearing the skin. The peripheral stimulation of loose skin about the head apparently initiates the reflex. It is possible that the presence of sand grains in the nasal aperture and about

the head may set off a similar reaction leading to dilation of the sinuses about the passages with attendant constriction of the nasal channels. (5) The narrowing of the nasal tubes would further impede the inspiration of sand grains.

Respiration itself is of such a nature that it favors the removal of sand from the nasal passages. (6) Inspiration is gradual. This has been concluded from observation of thoracic movements and the oscillation of sand particles over the nostrils. With inspiration the ribs move slowly outward and the grains of sand in the nostrils are scarcely disturbed. A small amount of material may be drawn into the nasal passages but with expiration the ribs move inward, at first rather slowly, later terminated with a vigorous contraction which drives the air forcibly from the lungs. The sand grains in the distal portion of the nasal tube are moved outward and the particles over the nostrils are forced away from the nasal opening.

The shape of the passages is such as to accentuate the effect of forceful expiration. The shape of a nasal passage when straightened out from the U-shaped configuration is roughly that of a cone. The expanded end is that which opens into the buccal cavity and corresponds to the principal nasal cavity. Air forced through the larger end of such a tube increases in velocity toward the constricted portion of the cone. The high velocity in this region carries away sand particles. Air drawn in with inspiration through the external naris rapidly decreases in velocity in the direction of the expanded portion of the cone resulting in deposition of sand grains. Most deposition occurs in the vestibular portion of the nasal passage which in part is due to gradual inspiration.

The lizard makes use of the previously described configuration in eliminating residual particles in the dorsal limb of the nasal tube. Upon coming to the surface, sand may be blown from the nostrils and the valve is usually depressed. Tongue action may be involved in compres-

sion of the air in the principal cavity. The air leaves the external naris with explosive velocity carrying with it any loose material which may be present.

As was pointed out in the description of the carbon particle experiment, under deeper soil conditions, funnels do not form over the nostrils. In other respects respiration is similar. Most of the facts previously brought out in connection with breathing under shallow soil conditions hold as well for respiration at greater depths.

On a number of occasions at both superficial and deeper levels specimens have been found to possess caps of encrusted sand over the nostrils. Sand grains become adherent apparently from moisture from the nasal tube. External conditions of humidity and temperature are associated with the degree of adherence of particles. Spaces occur between the agglutinated grains and there seems to be no interference with respiration. Under these conditions the freely movable, dry particles are prevented from entering the nasal tube by this grill of adhering fragments.

Up to this point attention has been largely directed toward the structure and function of the vestibule of the nasal passage with respect to subsurface respiration.

Dissection of a number of specimens has revealed that the lower portion of the nasal tube tends to remain free of sand. In a few cases a small deposit has been found, but in general the particles fail to pass around the abrupt bend in the nasal passage. If particles, though few in number, were allowed to accumulate indefinitely a sizable deposit would result and obstruction to breathing would ensue. It is not logically conceivable that these fragments which have reached the mucus covered epithelium of the principal cavity are blown out through the external naris. These grains are enveloped in mucus and are carried in such a film toward the internal naris.

The fluid secreted by Bowman's glands, which are located in the walls of the principal cavity, is augmented by the secretion from the lateral nasal gland which opens by two ducts just above the nasal cavity. The cilia beat

toward the internal naris and the sheet of mucus moved by their action conveys the particles of sand into the mouth and thence into the throat to be swallowed.

Sand in the digestive tract is not uncommon. It has been found in the stomach and in the faecal masses. Ingestion of grains often occurs in feeding. It is assumed that no ill effect would result from the swallowing of sand-laden mucus.

The glottis of *Uma* lies in a depression at the base of the fleshy tongue. The floor of the orbit projects inward on either side of the roof of the mouth cavity. Between the orbital projections there occurs a narrow furrow. When the mouth is closed the glottis is situated in this trough. The glottis is valvular. Two dorso-lateral wings joined at their upper edges swing inward ventrally to close the trachea. The presence of this valvular mechanism for closing the opening of the trachea acts as a final barrier against inspiration of foreign material.

CONCLUSION

Comparative studies reveal that certain other genera of the Iguanidae possess a similar type of nasal apparatus. The genera *Uta, Sceloporus, Phrynosoma* and *Callisaurus* are almost identical in structure to the genus *Uma*. At the same time there are genera of the Iguanidae which diverge considerably from the Uma-type. Examination of many other saurian families revealed no cases of similarity in olfactory configuration to the U-shaped passages of the preceeding genera of the Iguanidae.

Lubosch (1934) mentions that the vestibule of the olfactory organ may be an adaptation to the problem of removal of dust from inspired air, associated with the terrestrial habit. The amphibians have a very poorly developed approach tube. If this is true an exaggerated vestibular element of the nasal structure might logically be expected under conditions where the dust removal problem is greatly intensified. Such conditions would occur in subsurface respiration beneath fine, loose parti-

cles. In the Uma-like members of the Iguanidae this trend seems to have occurred. The olfactory chamber is essentially a long tube, the principal nasal cavity appears as a relatively small dilation. The displacement of the nasal cavity to a position ventral to the vestibule may be associated with the elongation within a limited space of the approach tube.

Most of the forms which possess a *Uma*-like nasal structure often submerge beneath finely divided soil. It is suggested that this tendency may be in part correlated with an efficient sand trap which facilitates respiration beneath loose material. The acquisition of the sand extracting mechanism doubtless was of great importance in the adoption of the almost completely psammophilous habit by the iguanid genus *Uma*.

Summary

Members of the iguanid genus *Uma* possess looped, U-shaped nasal passages which in structure and functioning prevent the inspiration of sand particles during subsurface respiration.

The following factors are involved in the exclusion and elimination of sand particles:

(1) The sand particles interfere with one another as they are drawn toward the external naris during inspiration. There is a tendency toward the formation of a grill of interlocking fragments which obstruct other loose grains.

(2) When *Uma* lies buried the vestibule is situated at an angle to the horizontal. The sand particles must be drawn up the approach tube against the pull of gravity before reaching the principal nasal cavity.

(3) Sand grains which adhere to the walls of the vestibule tend to impede the movement of free grains.

(4) Under conditions of subsurface respiration the valve at the external naris reduces the nasal aperture to a narrow crescentic slit, limiting the inspiration of particles.

(5) The postulated reduction in the diameter of the nasal tubes concomitant with the valvular action of the floor of the vestibule adjacent to the external naris, may tend to obstruct sand particles.

(6) Gradual inspiration coupled with explosive expiration aids in avoiding the accumulation of sand in the nasal tubes. The configuration of the olfactory channels accentuates the explosive effect of expiration.

(7) The small number of sand grains reaching the principal cavity are conveyed in a mucous film moved by ciliary action into the mouth, ultimately passing into the digestive tract.

The genus *Uma* is not unique with respect to nasal structure. A number of other genera of the Iguanidae were found to possess a nasal configuration closely similar to that occurring in this genus. *Callisaurus, Phrynosoma, Uta, Sceloporus* are such genera. A tendency toward soil submergence occurs among these groups as based on field and laboratory observation. Other non-burrowing[3] genera of the Iguanidae exhibited a nasal configuration quite different from the Uma-like types.

The occurrence of the looped nasal structure within the Iguanidae doubtless was of considerable importance in the development of the almost completely psammophilous habit of the genus *Uma*.

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[3] Burrowing is considered in the sense of submergence or "soil swimming" rather than in the sense of tunneling or the excavation of a cavity for retreat.

(EXPLANATION OF PLATES I AND II)

Natural size of specimen from which drawing was made measured 7 mm from posterior eye corner to tip of snout. Enlargement of drawing—× 7.

c.s. —ciliated surfaces
d.l.n.g. —duct of the lateral nasal gland
e.n. —external naris
i. —integument.
i.n. —internal naris
l. —lacrimal bone
l.n.g. —lateral nasal gland
m. —maxillary bone
n. —nasal bone
n.s. —nasal septum
n.v. —nasal valve
o. —orbit
pm. —premaxillary bone
p.f. —palatine fold
p.c. —principal cavity
t. —teeth
v. —vestibule

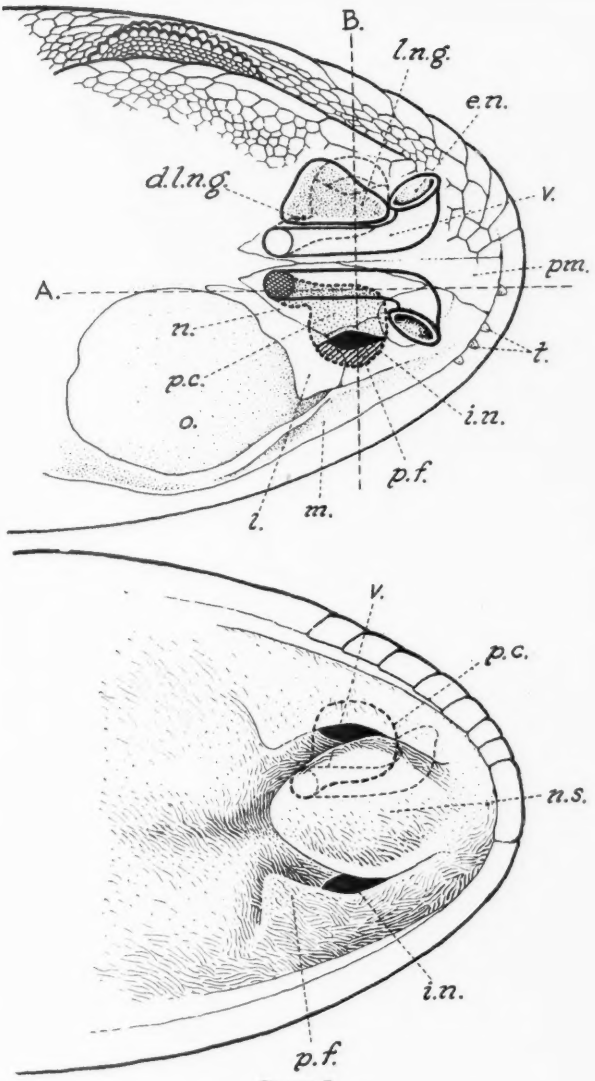

PLATE I

FIG. 1. Dorsal aspect of the head of *Uma inornata* showing, to the right of the mid-line, the relationship of the olfactory passages to the bones of the nasal region and to the left, the relationship of the nasal structure to the external morphology of the head. The lateral nasal gland has been omitted from the right side.

FIG. 2. Ventral view of the head of *Uma inornata* with the lower jaw removed. Dotted lines indicate the position of the nasal passage on one side.

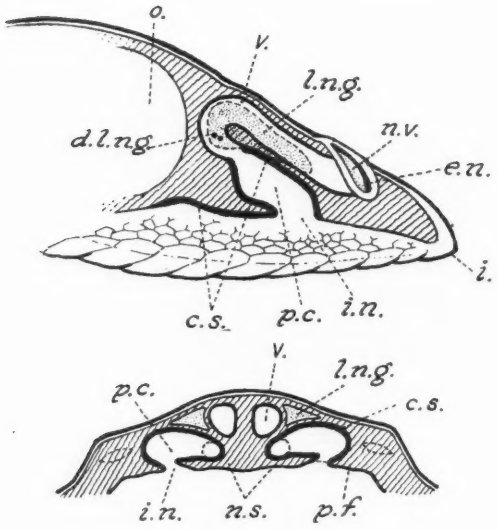

PLATE II

FIG. 1. Semi-diagrammatic parasagittal section through the nasal region of *Uma inornata* (see plate I, fig. 1, A). Heavy lines indicate ciliated surfaces. The drawing has been made to include the internal naris.

FIG. 2. Transverse section through the olfactory organs of *Uma inornata* (see plate I, fig. 1, B). Ciliated surfaces are indicated by heavy lines.

AN ESTIMATE OF THE MINIMUM NUMBER OF GENES DIFFERENTIATING TWO SPECIES OF GOLDEN-ROD WITH RESPECT TO THEIR MORPHOLOGICAL CHARACTERS

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IN the recent genetic analysis of evolution and speciation one relevant question of considerable interest from a taxonomic point of view seems to remain unanswered: in how many gene loci, on the average, are lines of descent differentiated at the stage when they are recognized as different species within a single genus? Theoretical studies of species formation have been focussed mainly on the kinetics of establishment, or rejection, of new genes at a *single locus* or within the set of loci relevant to a *single character*. Observational genetic studies of species differences have been mainly chromosomal, or concerned with proving that the visible differences have a mendelian basis, or with ascertaining the general type of gene action involved.

It also would be desirable to know, for at least a few pairs of species, about how many gene substitutions determine the *whole set of differentiating characters* recognized by taxonomists. It is likely that no very reliable answer can be obtained by present methods; and it is to be expected that no two species would give the same answer. However, these probable limitations can scarcely be evaluated or corrected without preliminary, crude attempts to estimate the number of taxonomically important gene differences between particular species pairs. Such an attempt is developed—incompletely—in the following pages, for two golden-rod species.

MATERIAL

Solidago rugosa Mill. is a meadow golden-rod with spreading inflorescence and inconspicuous basal leaf

rosette; *S. sempervirens* L. is a salt-marsh species, shorter than *rugosa,* with compact, secund inflorescence and conspicuous rosette. A specimen of each is shown

FIG. 1.

in Fig. 1. The differentiating characters are compiled in Table 1, taken from Goodwin (1937a).

The two species are not altogether isolated genetically. Where their ranges overlap—as in the reclaimed salt-

marsh near Cambridge, Mass., studied by Goodwin—a variety of intergrades is found. The intergrades can be duplicated in first- and second-generation artificial hy-

TABLE 1

MORPHOLOGICAL CHARACTERS DIFFERENTIATING *Solidago rugosa* FROM *S. sempervirens* (FROM GOODWIN, 1937a)[a]

	S. rugosa[b]	F_1 hybrid	*S. sempervirens*[b]
Natural habitat	Meadows, pastures, and roadsides	Dry borders of salt marshes, etc.	Beaches and salt marshes
Stems (at maturity)			
Height	(75–120) cm	(75–120) cm	(45–90) cm
Pubescence	Thick	Sparse	Absent
Cortex	Not fleshy	Not fleshy	Fleshy
Anatomy	Woody	Woody	Semi-herbaceous
Leaf traces	3	3	7–13[c]
Stolons	Long, spreading	Intermediate	Short, ascending
Leaves			
Length in rosette[d]	(5–12) cm	(10–35) cm	(15–60) cm
Shape	Ovate-lanceolate Narrowed at base Double-serrate	Lanceolate Intermediate Slightly serrate	Oblanceolate Base clasping Entire
Venation	Sub-pinnate	Intermediate	Sub-parallel
Texture	Not fleshy, veins prominent	Intermediate	Fleshy, veins not prominent
Hairs	Numerous, many-celled, linear, persistent; some flask-shaped hairs also present	Both types of hairs present; linear hairs scattered	3-celled, flask-shaped, deciduous (short, curved hairs sometimes on margin)
Length of stomatal apparatus	15 (10–24) μ	22 (15–29) μ	34 (25–46) μ
Cuticle	Not sculptured	Intermediate	Sculptured
Inflorescence			
Panicle	Racemes spreading, panicle broad	Intermediate and variable	Racemes short, panicle contracted
Head			
Involucre length	3.5 (3–4) mm	5.0 (3.6–5.5) mm	5.5 (4–6) mm
Bracts	Scarious except for midvein	Intermediate	Scarious only at margins
No. ray florets	8.5 (6–13)	10.5 (6–14)	11.5 (7–15)
No. disc florets	5.5 (3–7)	10.0 (7–16)	14.5 (11–20)
Total florets	14.0 (12–20)	20.5 (13–29)	26.0 (20–33)
% ray florets	61%	51%	44%
Ray floret length	(3.2–4.0) mm	(4.2–6.5) mm	(8.2–9.0) mm
Disc floret length	(3.0–3.5) mm	(3.0–4.5) mm	(5.0–5.7) mm
Pappus length	(2.0–2.8) mm	(3.0–4.2) mm	(4.5) mm
Fruit length	1.3 (1.0–2.0) mm	2.2 (1.5–2.6) mm	2.9 (2.0–3.6) mm
Fruit weight	0.13 mg	0.27 mg	0.80 mg

[a] Measurements were taken from numerous representative wild plants as well as from plants grown in the greenhouse. Figures represent the modes of each series of measurements. The extremes are indicated in parentheses.

[b] Varieties are not included.

[c] Excluding the cotyledons, which have three traces.

[d] Excluding the first eight seedling leaves and the early spring leaves of the older rosettes.

brids, as Goodwin showed. This indicates a very close relationship between these species, which some investigators may even prefer to reduce to subspecific rank.

The two species appear to be distinct, beyond the narrow range-overlaps, although no adequate study has been made of the extent to which the genom of one has been penetrated by genes of the other. Cytologically the two species seem to be quite alike: each has a haploid chromosome number of nine; the F_1 undergoes normal meiosis and forms normally viable pollen, although the germination of F_1 seeds is only three quarters as good as that of either parent (68 per cent against 90 per cent). Perhaps the "salt-marsh-physiology" genes are so intermingled throughout the chromosomes with "*sempervirens*-morphology" genes—and meadow-physiology with *rugosa*-morphology—that in either habitat the alien structure is continuously eliminated as a by-product of selection against alien function, the elimination balancing the cross-pollination in and near the zones of overlap. In any case, the number of places where the two species grow close enough together to render cross pollination probable are few and far between.

In establishing that the intergrades are natural hybrids, Goodwin (1937a) measured or classified 48 *rugosa*, 38 *sempervirens*, 56 F_1 and 108 F_2 plants, all greenhouse grown, with respect to six of the seven differentiating leaf characters. The results are shown in Table 2.[1]

CALCULATIONS

Genes acting on measured characters. The number of gene substitutions which determine a difference in some measurable character between two isogenic strains can be readily estimated (Wright, 1934) if numerous individuals of each strain and of their F_1 and F_2 have been measured with respect to that character. The method is indicated by the following formula, in which v_P and v_p represent the average measurements of parent strains, σ_1 and σ_2

[1] Of the F_1 plants, 20 have been omitted from the character 4 and 6 tallies because they were a year older than any of the other plants at the time of measurement; they have been included, however, in the tallies of the other four characters which are thought not to change with age of plant.

TABLE 2

PRINCIPAL FOLIAGE CHARACTERS IN *Solidago sempervirens*, *S. rugosa* AND THEIR HYBRIDS

Character:	1 leaf margin					2 leaf and stem surface					3 leaf thickness					5 leaf cuticle				
	entire				serrate	glabrous			pubescent		fleshy			not fleshy		sculptured			not sculptured	
Category:	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
sempervirens	38					38					38					34	4			
rugosa			1		47					48					48				3	45
F_1		3	53	1			3	53				3	53				5	46	5	
F_2	14	31	60	3		12	15	71	8	2	13	31	59	3	2	4	15	61	18	10

Character 4: length of basal leaves																										
Length (cm):	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	mean	standard deviation
sempervirens						1		1	2	3	3	4	3	2	2	2	4	2	3	3	1		1	1	17.8	...
rugosa		40		4	1	3																			5.5 ±	...
F_1				3	5	6	12	4	2	2		1				1									10.1	2.02
F_2	2	11	20	11	16	17	14	8	3	1	3	2													8.2	2.58

Character 6: length of stomatal apparatus																	
Length (μ):	12–13	14–15	16–17	18–19	20–21	22–23	24–25	26–27	28–29	30–31	32–33	34–35	36–37	38–	mean	standard deviation
sempervirens									1	4	11	9	11	2	34.4	2.49
rugosa	8	22	9	9											15.4	1.82
F_1				2	23	10	1								21.1	1.07
F_2			3	12	22	26	15	13	6	3	5	1	1	1	23.6	4.16

the standard deviations of F_1 and F_2, and n the number of gene differences:

$$n = \frac{(v_P - v_p)^2}{8(\sigma_2^2 - \sigma_1^2)}.$$

For example, the average length of stomatal apparatus in *S. rugosa* is 15.4 μ, in *S. sempervirens* 34.4 μ. The standard deviations of F_1 and F_2 are 1.07 μ and 4.16 μ. So the estimated number of gene differences relevant to stoma length, between the two species, is $(34.4 - 15.4)^2/8(4.16^2 - 1.07^2)$ or 3, to the nearest integer.

Wright's formula gives a *minimal* estimate, as he points out. It is derived on the assumptions that (1) each strain is isogenic with respect to the character studied, (2) the large-size genes are concentrated in one strain, the small-size genes in the other, (3) the genes involved have equal effects, without dominance or epistasis, and (4) no two of the loci are in the same chromosome. If these assumptions do not match the actual conditions in material to which the method is applied, the estimated number of gene differences will be smaller than the actual number. To what extent the assumptions are valid in the present case will be discussed below; whatever the answer may be, we shall at least have a minimal estimate.

Genes acting on categorized characters. Wright's method can also be applied to such characters as 1, 2, 3 and 5 (Table 2) with respect to which individuals have been grouped into broad categories, rather than measured (Wright, 1934). For this purpose it is assumed that successive categories represent successive ranges, not necessarily equal, of a normal distribution of values in any particular generation. The value of the boundary between two contiguous categories can then be obtained, in terms of the mean and standard deviation of the generation, by entering a table of areas under the normal curve with the proportion of the group in all categories above (or below) the desired boundary. As an example we may go in detail through the analysis of character 5, degree of sculpturing of leaf epidermis.

	numerical value of boundary, T, between categories			
	1 and 2	2 and 3	3 and 4	4 and 5
	$T_{1.2}$	$T_{2.3}$	$T_{3.4}$	$T_{4.5}$
sempervirens	$v_s + 1.25\sigma_s$			
rugosa				$v_r - 1.54\sigma_r$
F_1		$v_1 - 1.46\sigma_1$	$v_1 + 1.24\sigma_1$	
F_2	$v_2 - 1.78\sigma_2$	$v_2 - 0.93\sigma_2$	$v_2 + 0.64\sigma_2$	$v_2 + 1.32\sigma_2$

Hence $(v_r - v_s) = (T_{4.5} - T_{1.2}) + 1.25\sigma_s + 1.54\sigma_r = (T_{4.5} - T_{1.2}) + 2.79\sigma_1$

If $\sigma_r = \sigma_s = \sigma_1$

$$(T_{3.4} - T_{2.3}) = (1.24 + 1.46)\sigma_1 = (0.64 + 0.93)\sigma_2, \text{ so } \sigma_2 = \frac{2.70}{1.67}\sigma_1 = 1.62\sigma_1$$

$$(T_{4.5} - T_{1.2}) = (1.32 + 1.78)\sigma_2 = 3.10\sigma_2 = 3.10 \times 1.62\sigma_1 = 5.01\sigma_1$$

so $(v_r - v_s) = (2.79 + 5.01)\sigma_1 = 7.80\sigma_1$, and

$$n = \frac{7.80^2\sigma_1^2}{8(1.62^2 - 1^2)\sigma_1^2} = 5 \qquad \text{(to the nearest integer)}$$

The same method, with one modification, can be applied to the remaining categorized characters: where there are individuals on only one side of a boundary whose value is essential (*e.g.*, character 2, F_1, 3–4 boundary) it is assumed that the boundary is 2.5 σ from the group mean.

Total gene differences relevant to leaf characters. The significant statistics for each of the six leaf characters recognized as differentiating *S. sempervirens* from *S. rugosa,* and the corresponding estimates of gene differences, are shown in Table 3.

TABLE 3

STATISTICS FOR DETERMINATION OF MINIMAL TOTAL GENE DIFFERENCES, RELEVANT TO LEAF CHARACTERS, BETWEEN *Solidago sempervirens* AND *S. rugosa*. Δ IS THE DIFFERENCE IN AVERAGE MEASUREMENT BETWEEN SPECIES DIVIDED BY THE STANDARD DEVIATION OF F_1; R IS THE STANDARD DEVIATION OF F_2 DIVIDED BY THAT OF F_1; $n = \Delta^2/8(R^2-1)$, TO THE NEXT INTEGER ABOVE THE EXACT VALUE

Character	Δ	R	Minimal number of gene differences n
1. leaf margin: entire to serrate	11.0	1.81	7 (6.6)
2. leaf surface: glabrous to pubescent ...	11.8	2.06	6 (5.2)
3. leaf thickness	11.7	2.06	6 (5.3)
4. basal leaves: length	6.1	1.28	8 (7.4)
5. leaf cuticle: degree of sculpturing	7.8	1.62	5 (4.7)
6. stomatal apparatus: length	17.8	3.89	3 (2.6)
		Total	35

It is of some interest to note that here, as in so many previous and more reliably studied cases, even the probably unimportant morphological details such as serration

of leaf margin are controlled by genes in many or most of the chromosomes. Of course, only a very small part of the genetic control of each character is detected, namely, that part which is unlike between two species which are certainly very closely related.

The *total* leaf-differentiating loci need not be as numerous as 35, the total of Table 3. Length and thickness of leaves and size of stomatal apparatus are all "size characters," and so are perhaps determined to some extent by the same genes. Each gene with effects on more than one of the characters studied would contribute to the F_2 variation of each character, and so would be included in Table 3, once for each effect.

How many genes with manifold effects are represented here can be estimated by a method which involves assumptions 1, 3 and 4 of Wright's formula (*cf.* page 58 of this paper). The following quantities are used:

Δ_x, the difference between parents in average value of character x, divided by the standard deviation of that character in F_1, *i.e.*, $\Delta_x = (x_P - x_p)/\sigma_{x1}$;
Δ_y, the analogous quotient for character y, *i.e.*, $\Delta_y = (y_P - y_p)/\sigma_{y1}$;
R_x, the quotient of standard deviations of character x in F_1 and F_2, *i.e.*, $R_x = \sigma_{x2}/\sigma_{x1}$;
R_y, the analogous character-y quotient, *i.e.*, $R_y = \sigma_{y2}/\sigma_{y1}$;
r_1, the correlation between characters x and y in F_1;
r_2, the correlation in F_2;
n_C, the number of genes acting on both characters;
n_L, the number of character-x loci each linked with a character-y locus;
c, the average crossover value between linked pairs of genes.

This method leads to the following formula:

$$n_L(1-2c) + n_C = \frac{\Delta_x \Delta_y (r_2 R_x R_y - r_1)}{8(R_x^2 - 1)\,(R_y^2 - 1)}$$

The quantities in the right-hand member of the equation, except the correlation coefficients, are those of Table 3. The correlation coefficients, calculated from the data, are taken as positive, whatever their actual sign may be.

For length of leaves and of stomatal apparatus

$$n_L(1-2c) + n_C = \frac{6.1 \times 17.8 (0.348 \times 1.28 \times 3.89 - 0.183)}{8(1.28^2 - 1)\,(3.89^2 - 1)} = 2.33$$

Therefore it seems relatively improbable that all three stoma genes also affect leaf size, since n_C could not be 3

unless $n_L(1-2c)$ were negative, which is meaningless. Three possibilities are left, any of which satisfies the condition that there are three stoma gene differences altogether:

Number of common genes	Number of linked pairs	Average crossover value (c)	Total stoma and leaf length genes
2	1	33%	9
1	2	17%	10
0	3	11%	11

Which of these possibilities is correct can not be determined, since they all meet the only available condition, that $n_L(1-2c)+n_C$ should have a value of 2.33. Now if it is recalled that 3 is a *minimal* estimate of stoma-length genes, and 8 a *minimal* estimate of leaf-length genes, it becomes obvious that the comparable limiting value for leaf and stoma jointly is that of *maximal common genes*, 2, and of *minimal total genes*, $3+8-2=9$. In general, then, the possibility of linked gene pairs need not be considered further, and attention can be confined to common genes, of which the maximal estimate is the next integer below the value of $n_L(1-2c)+n_C$ for any particular character pair.

The calculated values of $n_L(1-2c)+n_C$ for each of the fifteen pairs among the six leaf characters studied are shown in Table 4, with the correlation coefficients upon which they depend. The correlation coefficients were calculated from the original data (unpublished). The maximal common gene estimates are shown in Fig. 2.

The interrelationship, shown by Fig. 2, within the genom which is responsible for the distinguishable leaf differences between *Solidago sempervirens* and *S. rugosa* might seem almost hopelessly complicated. But in fact an answer is readily obtained to the one question under consideration here: what is the *smallest* number of gene substitutions which will account for the phenotypic differences? No two among the characters 1, 4 and 5 show any evidence of common genes (common gene indices,

in Table 4, 0–1; estimates of maximal common genes, in Fig. 2, 0). Hence the differences in serration, length and sculpturing altogether *can not* be accounted for by a number of gene differences less than the sum of the substitutions affecting the characters separately, *i.e.*, $7 + 8 + 5$ or 20.

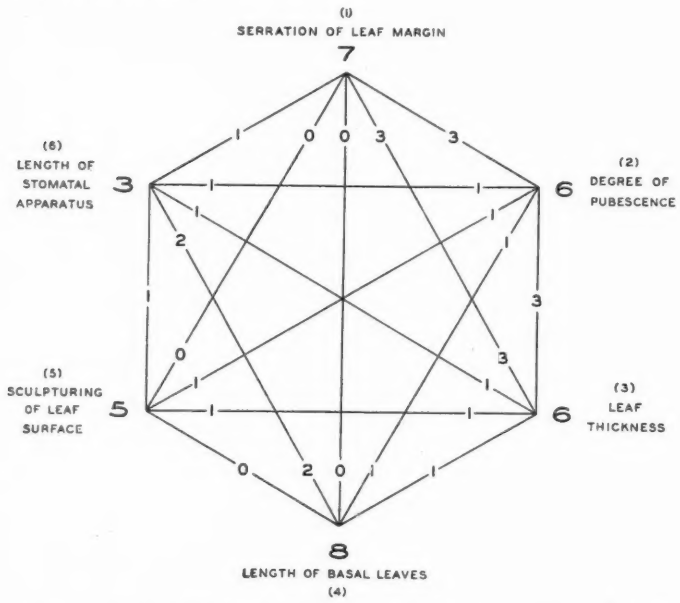

FIG. 2. Leaf characters differentiating *Solidago sempervirens* from *S. rugosa*, estimated minimal number of gene differences determining each character difference (large digits at apices of hexagon), and maximal number of genes acting on both members of each possible character pair (small digits on lines connecting characters).

Some among the 20 might also account for the stoma-length difference, since Fig. 2 shows that stoma length and epidermal sculpturing may be affected in common by one gene, stoma and leaf length by two, stoma length and marginal serration by one. The potential total, four, is greater than the number of stoma genes to be accounted for, three. Of course it is possible that one or more of the stoma length genes is in fact different from any of the 20

which control characters 1, 4 and 5. In this case the total controlling the differences in characters 1, 4, 5 and 6 altogether would be greater than 20, which is the least possible total.

Character 3, leaf thickness, may be affected in common with leaf serration by as many as three genes; with epidermal sculpturing, by one; with leaf length, by one. The potential maximum of 5 genes in common with the pre-

TABLE 4

Correlations between pairs of taxonomically significant leaf characters in F_1 and F_2 hybrids between *Solidago sempervirens* and *S. rugosa*, with an index which is equal to the number of genes acting on both characters *plus* a function of the number of linked gene pairs (one gene of each pair affecting one character, one the other). The characters involved are: 1, serration of leaf margin; 2, degree of pubescence; 3, thickness; 4, length of basal leaves; 5, sculpturing of leaf surface; 6, length of stomatal apparatus.

Character pair	Correlation* in F_1	Correlation* in F_2	Common gene index
1.2	+.071	+.448	3.5
1.3	−.008	+.457	3.7
1.4	−.087	−.066	0.4
1.5	0	+.038	0.3
1.6	+.066	−.271	1.4
2.3	+.068	+.514	3.5
2.4	−.059	−.163	1.6
2.5	0	+.199	1.5
2.6	+.028	−.272	1.2
3.4	−.067	−.176	1.7
3.5	0	+.136	1.0
3.6	+.136	−.399	1.7
4.5	−.103	−.073	0.3
4.6	+.183	+.348	2.4
5.6	−.101	−.350	1.6

* In calculating the coefficients which involve characters 1, 2, 3 and 5 the class values of Table 2 were transformed as in Pearson's broad categories correlation method. *But* the standard deviations of the categorized characters were taken as one rather than the quantity given by the transformed class values. This procedure was followed because the F_1 data are so scanty that the calculated standard deviations are in some cases considerably affected by the classification of one or a few individual plants. The procedure underestimates the true correlations, more so in the F_1 than in the F_2; the errors in the two generations have opposite, and so at least partly compensating, effects on the value of the common gene index.

viously considered characters leaves one of the six thickness genes unaccounted for. So the total phenotypic differences in characters 1, 3, 4, 5 and 6 altogether must depend on at least 20 plus 1 gene substitutions. These 21 will also account for the genetic control of differences in pubescence which may have three genes in common with serration, one with sculpturing, one with leaf length and three with leaf thickness.

Altogether then, the six distinguishable leaf differences

between *Solidago sempervirens* and *S. rugosa* might be determined by as few as 21 gene substitutions. That the estimation method leaves open the possibility—indeed, the probability—of more than 21 should perhaps be recalled here. But there can scarcely be fewer. A number less than 21 would not account for the observed differences in standard deviations and correlations between F_1 and F_2 from which the estimates of Table 4 and Fig. 2 are derived.

In criticism of this conclusion it might well be pointed out that the statistics upon which the estimates depend, and therefore the estimates themselves, are subject to large sampling errors because the populations measured were so small. Furthermore, the estimation methods are strictly applicable only to crosses between isogenic strains, while the P_1 plants of the present crosses were grown from the seeds of wild plants or were only one generation removed from wild seeds. To these criticisms no answer seems possible except that suggested above, that a very crude attempt at the problem is perhaps better than none at all.

The criticisms might arise that the estimations are aimed at the minimal, rather than the most probable, number of gene differences, at readily recognizable phenotypic contrasts, rather than the whole array; and that no consideration is given to the inevitable complex linkage relations within a set of 21 loci distributed among 9 chromosomes. The answer here is obviously one of what is practicable without endless mendelian and physiological study.

An estimate of total gene differences relevant to all the morphological characters. A minimum estimate has now been made of the number of genes involved in differentiating *S. sempervirens* from *S. rugosa* with respect to six obvious morphological leaf characters. The question posed earlier in this paper as to what may be the minimum number of genes producing all the obvious morphological differences between these two species still

remains unanswered. Reference to Table 1 will show that other characters may be found in the stems, leaves and inflorescence. To what extent would an analysis of these other characters be likely to increase our minimum estimate of gene number?

It is well known that certain genes may have a general effect upon the development of several different parts of an organism, as in the case of a number of genes in *Primula sinensis* (de Winton and Haldane, 1933). In a recent analysis of the specific differences between two species of *Nicotiana,* Anderson and Ownbey (1939) have pointed out that these differences may be grouped into categories which they call genetic coefficients. The inference is that a single gene or a battery of genes controlling the expression of a morphological character in one structure may control the development of a similar character in another type of structure in the same plant. Differences in one or more genes within such a battery might then account for morphological differences in various parts of the plants of the two related species. In *Nicotiana* crosses, for example, Anderson (1939) reports a close positive correlation between the length of style and the magnitude of various corolla measurements. There is no certainty, however, that a given set of genes will always have similar morphological effects in all parts of an organism. Indeed, this has been shown not to be the case in certain instances.

Regardless of this criticism, in order to make an estimate of the *minimum* number of genes differentiating the golden-rod species under consideration, this principle of "genetic coefficients" should be applied. If the assumption should prove to be incorrect in any particular case it would mean increasing our estimate. Hence the various characters in Table 1 have been regrouped under the following headings according to their possible genetic association and the nature of the association is discussed briefly in each case.

(1) *Organ size.* In general, most of the organs of *S.*

sempervirens are larger than those of *S. rugosa.* A developmental analysis of the leaves of these two species (Goodwin, 1937b) has shown that greater cell number and greater cell size in the lamina and along the midrib, and prolonged meristematic activity near the base of the petiole, are responsible for the larger total size and longer petiole of *S. sempervirens.* Hence the genetic control of leaf size (and at the same time of leaf shape) must be most complex in this material. The previous analysis of character 4, leaf length, indicates that at least 8 genes are contributing toward the differentiation of the two species with respect to this character, while size differences in the stomatal apparatus, character 6, are controlled by at least three genes not more than two of which are common to the leaf-size set.

In the inflorescence, all the floral parts are also more or less proportionately larger in *S. sempervirens* than in *S. rugosa;* involucral bracts, receptacle, ray florets, disc florets, pappus, fruit, etc. The total number of florets is probably dependent upon the size of the receptacle; and the proportion of ray to disc florets may also depend upon receptacle size, since ray florets are in a marginal position in the head and since the margin of the receptacle increases directly with its diameter, while the area increases as its square. In estimating the minimal number of genes involved, the assumption might be made that the same set of genes operates both in the leaves and in the reproductive structures in determining these size differences.

(2) *Length of axes.* The length of the main axis of the shoot, of the lateral branches of the inflorescence, and of the underground stolons is greater in *S. rugosa* than it is in *S. sempervirens.* These are the only structures which are larger in this species. No genetic data are available on this series of size characters. It is entirely possible that they are all differentiated at least in part by a common set of genes.

(3) *Parenchyma.* The stem cortex and leaf mesophyll

are developed to a greater extent in *S. sempervirens* than they are in *S. rugosa.* This gives both of these structures a more fleshy, succulent appearance in the former species. The genetic analysis of leaf thickness, character 3, indicates that at least 6 genes are involved. These may also influence the development of the cortex. It is suggested that this same set of genes may be responsible for the lack of scarious margins in the bracts of *S. sempervirens* as well.

(4) *Leaf traces.* In *S. rugosa* and the F_1 hybrid the leaf traces are three in number. In *S. sempervirens* the number ranges from 7 to 13. The leaf traces connecting the vascular system of the stem with that of the leaf leave gaps in the vascular cylinder above their point of departure. The large number of these gaps in *S. sempervirens* contributes toward the herbaceous nature of the stem in this species. Furthermore, the traces going to a given leaf arise from points all around the stem and pass directly into the clasping base of the petiole as parallel veins. No genetic analysis of leaf trace determination has been made. The nature of the stem, the leaf base and the leaf venation might all be characters affected by this factor.

(5) *Leaf margin.* The leaves of *S. rugosa* are serrate while those of *S. sempervirens* are entire. The nature of the leaf margin, character 1, appears to be controlled by at least 7 genes.

(6) *Pubescence.* Epidermal hairs are of two sorts in these species. Linear, multicellular hairs are present in *S. rugosa,* but not in *S. sempervirens.* Six genes are apparently involved in determining this character difference. Flask-shaped, deciduous hairs are present in *S. sempervirens,* but not in *S. rugosa.* In this case no genetic analysis is available. The character is not of much practical importance to the taxonomist, since these hairs are usually absent from mature leaves and from herbarium specimens.

(7) *Cuticular surface.* In the leaves of *S. semper-*

virens the cuticle is sculptured into many close furrows, whereas the cuticle of *S. rugosa* is smooth. In this case, character 5, our analysis indicates a minimum of 5 genes in operation.

It has already been estimated that a minimum of 21 genes is responsible for the morphological differences between the leaves of *S. rugosa* and *S. sempervirens,* a battery of genes being responsible for the differentiation of each character. The foregoing discussion lays the basis for the assumption that certain other characters in the stem and inflorescence may be differentiated by these same batteries. Characters which may well be differentiated by sets of genes different from and comparable in number to any included in our previous estimate are the length of the axes, the number of leaf traces and the presence of flask-shaped hairs. Hence it might be legitimate to increase our estimate by a factor of two, giving us about 40 gene substitutions responsible for all the obvious morphological differences between these two species. Any genes common to the unstudied characters and to the leaf characters would reduce this figure. We realize that this figure is at best a very crude minimum estimate. It should be pointed out again that the actual number of genes involved is probably much larger than this minimum value.

A similar but more exhaustive analysis on more suitable genetic material such as the *Nicotiana Langsdorffii-N. alata* cross, which has already been studied in considerable detail, is greatly to be desired.

SUMMARY

The differences between the leaf characters of two species of golden-rod, *Solidago rugosa* and *S. sempervirens,* have been shown to be determined by a minimum of 21 genes. It is pointed out that a minimum of about twice as many genes are probably in operation in the control of all the morphologically distinguishable differences between these two species.

A method has been devised to estimate the maximum number of gene differences common to two morphological character differences between related strains or interfertile species from the standard deviations and correlations of the character pairs in the F_1 and F_2 hybrids.

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THE LINKAGE OF POLYDACTYLY WITH MULTIPLE SPURS AND DUPLEX COMB IN THE FOWL[1]

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FOLLOWING discovery of a fifth autosomal linkage group in the fowl, containing the genes, *D*, for duplex comb, and *M*, for multiple spurs (Hutt, 1941), further tests were made for linkage of other genes with these two.

LINKAGE OF *M* AND *Po*

The studies already reported showed *M* to be independent of the other four autosomal linkage groups, and it was therefore desired to test it and *D* with other mutations known not to belong to those groups. A test of four such genes was easily arranged by mating black *dd MM* females with a Mottled Houdan male and backcrossing to Anconas. The F_1 heterozygotes were of the constitution $\frac{D\ m\ Cr\ Mu\ Po\ W\ e'}{d\ M\ cr\ mu\ po\ w\ E^+}$, carrying the four mutations, *Mu* (muffs), *Po* (polydactyly), *W* (white skin) and *e'* (mottling), which had not been assigned to any known linkage group, although all but mottling had been used in several linkage tests. These birds were all heterozygous for crest (*Cr*), which was not needed in this study because *M* had already been proven independent of the linkage group (*F I Cr*) to which crest belongs. Some of these linkage testers also carried the gene *Fl* (flightless) previously shown to be not linked with *M*.

When the multiple heterozygotes were backcrossed to Anconas, which carry the recessive alleles of all the mutations mentioned above, *Mu*, *W* and *e'* showed independent

[1] No. 16 in the author's series "Genetics of the Fowl."

assortment with *D* and *M* and with each other. However, *Po* proved to be linked with *m*, there being 33.5 per cent. crossing over in 514 gametes.

Polydactyly is not an ideal mutation for linkage studies because it is suppressed in many heterozygotes. Backcrosses yielding clear 1 : 1 ratios are not usual. Because polydactyly has previously been assigned by Dunn (1927) and Dunn and Landauer (1930) to the *F I Cr* chromosome, and also because it has been used in many unsuccessful tests for linkage, the present evidence for its linkage with *M* and *D* is given in full. This comes mostly from progeny of ten females mated to an Ancona male, H 5100, in the spring of 1941. Classifications of these chicks at hatching are shown in Table 1. In most cases these were verified at later ages, although this was not essential because polydactyly is easily recognized in the day-old chick, and, as was previously shown (Hutt, 1941), classifications of multiple spurs at that age are almost completely accurate.

TABLE 1

EVIDENCE FROM BACKCROSSES THAT POLYDACTYLY, *Po*, IS LINKED WITH MULTIPLE SPURS, *M*

Heterozygotes tested	Classification of progeny				Gametes tested		Crossing over
	Parental classes		Crossovers		Total	Crossovers	
	Po m	*po M*	*Po M*	*po m*	*number*	*number*	*per cent.*
♀ 4251	19	31	12	16	78	28	35.9
4257	26	23	13	16	78	29	37.2
4259	8	13	8	8	37	16	43.2
4261	35	24	11	14	84	25	29.8
4262	16	23	9	9	57	18	31.6
4272	23	24	10	15	72	25	34.7
4255	11	7	4	6	28	10	35.7
4260	13	10	9	2	34	11	32.4
4263	9	6	1	2	18	3	16.7
4266	13	8	1	6	28	7	25.0
Total	173	169	78	94	514	172	33.5

In the 514 birds from this mating, the ratio of *Po* : *po* was 251 : 263, while that of *M* to *m* was 247 : 267. Expressed otherwise, polydactyly was present in 98 per cent. of the number expected (257) and multiple spurs in 96 per cent. Since the deviation from expectation was so small with both mutations, it follows that there was little or no suppression of *Po* or *M*, and hence that the data are

suitable for the measurement of linkage. The composite figure of 33.5 per cent. crossing over is reasonably reliable, not merely because it was measured in 514 gametes, but also because these came from ten different birds, among which some variability in the amount of crossing over was to be expected. This figure was substantiated by another backcross in which a male heterozygous for *M* and *Po* was mated with doubly recessive females. Sixty per cent. of the 98 progeny were eliminated from consideration because manifestation of *Po* in their sibships was considerably below the expected 50 per cent. However, in the remaining 39 chicks, comprising families with little or no suppression of *Po*, the number of new combinations was 14. The amount of crossing over in this male—35.9 per cent.—was therefore quite close to the 33.5 per cent. measured in gametes of the ten females listed in Table 1.

LINKAGE OF *Po* OBSCURED BY ITS SUPPRESSION

These studies provided a good example of the way in which linkage tests involving polydactyly (or other genes) might be incorrectly interpreted whenever the character is not manifested in all heterozygotes. The last four females listed in Table 1 were originally bred to another Ancona male, H 4999, that apparently carried modifying genes suppressing both *Po* and *M*. In his 118 progeny, the ratio of *Po* to *po* was 24 : 94, and of *M* to *m*, 34 : 84. With 59 expected in each class, it is clear that polydactyly was suppressed in about 60 per cent. of the birds heterozygous for it, and multiple spurs in 42 per cent. In striking contrast to this, the mating of these same four hens with ♂ H 5100 yielded an excess of polydactylous offspring above the 50 per cent. expected and a slight deficiency of multiple-spurred birds no greater than could have occurred by chance.

These diallel crosses show nicely (1) the difficulty of measuring linkage in matings where *Po* is suppressed and (2) the validity of linkage tests where manifestation of

TABLE 2

DEMONSTRATION BY DIALLEL CROSSES OF DISTORTED RATIOS AND CONCEALMENT OF LINKAGE CAUSED BY SUPPRESSION OF TWO LINKED CHARACTERS

Mating of four females	Classification of progeny				Gametes tested	Crossing over
	Parental combinations		New combinations			
	Po m	*po M*	*Po M*	*po m*	*number*	*per cent.*
(a) With ♂ H 5100	46	31	15	16	108	28.7
(b) With ♂ H 4999	21	31	3	63	118	55.9
Expected with 33.5 per cent. crossing over*	19.4	29.4	4.6	64.6	118	33.5
Expected with no linkage* .	17.1	27.1	6.9	66.9	118	none
Expected with no linkage and no suppression	29.5	29.5	29.5	29.5	118	none

* When corrected on the basis that *Po* is suppressed in 59 per cent. and *M* suppressed in 42 per cent.

that character is not inhibited (Table 2). The mating with ♂ H 5100 revealed 28.7 per cent. crossing over between *Po* and *M*, a figure not out of line with the 34.7 per cent. measured in gametes of the other six hens in the same mating. In contrast to this, progeny of ♂ H 4999 showed an apparent crossing over of 55.9 per cent., which would ordinarily be interpreted as indicating the independence of *Po* and *M*. Suppression of these two genes reduced the *Po M* class to 3 and increased the double recessives to 63. When the expected ratio is calculated after making allowance for the suppression of *Po* and *M* and for 33.5 per cent. crossing over (Table 2), a close fit of the numbers observed to those expected in each class is obtained ($\chi^2 = 0.85$). Even the deviation of the observed ratio from that expected with the same allowance for suppression of the two genes but with no linkage is only slightly greater than could occur by chance ($\chi^2 = 3.91$, $p = .05$). This is because the expected 1 : 1 : 1 : 1 ratio is distorted more by suppression of *Po* and *M* than by crossing over.

It is evident that tests for linkage of *Po* can be utilized whenever that character segregates in a clear 1 : 1 or 3 : 1 ratio but are subject to suspicion when it does not.

ARRANGEMENT OF *D*, *M* AND *Po* IN THE CHROMOSOME

In the previous report, Hutt (1941) showed that *D* and *M* are linked with 28 per cent. crossing over between

them. Since there is 33.5 per cent. crossing over between *M* and *Po*, it follows that *Po* must be either to the left of *D*, and very close to it, or so remote from *D* as to appear almost independent. That the latter alternative is the correct one is shown by the following data for 517 progeny of the ten females listed in Table 1, each of which carried *D* and *Po* in the coupling phase:

Parental combinations		New combinations		Crossing-over
D Po	*d po*	*D po*	*d Po*	per cent.
number	number	number	number	
143	157	108	109	42

Since there was in this population almost complete manifestation of polydactyly (252 *Po* : 265 *po*) and of duplex comb (251 *D* : 266 *d*), the 42 per cent. crossing over can not be questioned. The relationship of the three genes is therefore approximately *D* 28 *M* 33 *Po*.

The relation of *D* to *Po* had previously been examined by the senior author, but, since in his (unpublished) data for 631 gametes tested the manifestation of *Po* was only 59 per cent. of the number expected, the test was of doubtful value and no conclusions were drawn. (Actually, when the crossovers between two linked genes approach 50 per cent., as in this case, the distortion of ratios by suppression of one of the linked genes causes little error in the measurement of linkage. This is because the number of zygotes converted by such suppression from a parental to a crossover class is almost balanced by the reciprocal conversion.) Similarly, the data of Serebrovsky and Petrov (1930), who found 31 per cent. crossing over between *D* and *Po* in one test, were hardly conclusive because in two others the amounts of apparent crossing over were 53 and 54 per cent. In these two matings, manifestation of one or both of the two dominant alleles was poor.

Double crossing over. The constitution of ♂ H 5100 with respect to the three linked genes was $\frac{D\ m\ Po}{d\ M\ po}$. It would be expected that, when the cumulative "distance"

between *D* and *Po* was 61 per cent., there should be some double crossing over between these two genes. The number of such cases observed was 48, or 9.3 per cent., which, oddly enough, was exactly the number expected $\left(\frac{28 \times 33.5}{100}\right)$. Of these, 17 were *D M Po* and 31 *d m po*. The amount of apparent crossing over between *D* and *Po* expected on this basis was 61—(2 × 9.3), or 42.4 per cent., a figure almost identical with the 42 per cent. actually observed.

Modifying Genes

The diallel crosses of four females heterozygous for both *M* and *Po* to two different males (Table 2) suggest that modifying genes preventing the appearance of polydactyly in heterozygotes also inhibit the manifestation of multiple spurs. In 108 progeny of H 5100, the proportion with *Po* was 56 per cent., and with *M*, 43 per cent., both figures being reasonably close to the expected 50 per cent. In contrast, among 118 progeny of these same females by ♂ H 4999, only 20.3 per cent. exhibited polydactyly and 29 per cent. had multiple spurs.

Similarly, in another mating in which an *Mm Po po* male was backcrossed to doubly recessive females, the number of progeny with the dominant allele was only 74 per cent. of that expected for polydactyly and 88 per cent. of the number expected with multiple spurs. Although it seems likely that modifiers suppressing polydactyly tend also to inhibit multiple spurs, the effects are not equal upon both mutations, and different modifying genes may be involved. From previous studies it is clear that polydactyly is suppressed more often than multiple spurs. It is perhaps to be expected that both mutations would be affected by the same modifying genes, since the areas involved are in close proximity and the effects of the two genes are somewhat alike. *Po* causes duplication of one or more phalanges of the first toe, while *M* modifies several scales and induces from three to five centers of ossification where only one is normal. Although these

processes are not identical, they have some features in common.

GENES INDEPENDENT OF THE *D M Po* CHROMOSOME

For the convenience of other investigators, there is summarized in Table 3 the evidence previously published and some new data to show which genes are independent of this latest linkage group to be discovered in the fowl. Some of the studies with multiple spurs were reported previously. Other tests of *M* with mottling, muffs and beard and white skin are omitted from Table 3 because, since these last three mutations are independent of *D* and

TABLE 3

CHARACTERS APPARENTLY INDEPENDENT OF THE *D M Po* GROUP

Linked gene	Tested with	Investigator		Back-cross ratio*
	Cp R U group			
D	Rose comb	Serebrovsky and Petrov	(1930)	61 : 55
"	Creeper	Landauer	(1932)	145 : 141
Po	Creeper	Serebrovsky and Petrov	(1930)	99 : 91
"	Rose comb	" " "	(1930)	417 : 391
"	Rose comb	Warren	(1933)	148 : 146
	F I Cr group			
D	Frizzling	Warren and Hutt	(1936)	172 : 150
"	Crest	Serebrovsky and Petrov	(1930)	225 : 203
"	Crest	Warren and Hutt	(1936)	409 : 389
Po	Frizzling	" " "	(1936)	163 : 166
"	Crest	Serebrovsky and Petrov	(1930)	160 : 130
"	Crest	Warren	(1933)	121 : 113
	O P Ma group			
D	Pea comb	Serebrovsky and Petrov	(1930)	123 : 104
Po	Pea comb	" " "	(1930)	152 : 160
	h Fl group			
D	Flightless	Hutt and Mueller		192 : 183
M	"	Hutt	(1941)	120 : 122
	Other mutations			
D	Blue	Serebrovsky and Petrov	(1930)	47 : 39
"	Recessive white	" " "	(1930)	13 : 13
"	Extension of black	" " "	(1930)	276 : 219
"	Mesodermal pigment	" " "	(1930)	143 : 128
"	Muff and beard	" " "	(1930)	241 : 226
"	Muff and beard	Hutt and Mueller		367 : 344
"	Naked neck	Landauer	(1932)	102 : 104
"	White skin	Serebrovsky and Petrov	(1930)	62 : 61
"	White skin	Hutt and Mueller		138 : 142
"	Mottling	" " "		324 : 328
Po	Blue	Serebrovsky and Petrov	(1930)	162 : 171
"	Extension of black	" " "		250 : 241
"	Feathered legs	Warren	(1933)	199 : 182
"	Mesodermal pigment	Dunn	(1927)	41 : 47*
"	Muff and beard	Serebrovsky and Petrov	(1930)	143 : 144
"	Muff and beard	Hutt and Mueller		345 : 367
"	Naked neck	Serebrovsky and Petrov	(1930)	144 : 182
"	Naked neck	Warren	(1933)	187 : 239
"	Rumpless	"	(1933)	199 : 181
"	White skin	"	(1933)	112 : 110
"	White skin	Hutt and Mueller		150 : 130
"	Mottling	" " "		328 : 326

* Ratios given are those of parental combinations to new combinations in back-crosses, except for Dunn's figures, which are from an F_2 population.

Po, at the opposite ends of the linkage group, they must also be independent of *M* in its center. Similarly, it is unnecessary to give the extensive evidence that *D*, *M* and *Po* are independent of dominant white so long as these three are shown to be independent of frizzling and crest, which are at opposite ends of the group to which dominant white belongs.

Undated tests ascribed to the present authors refer to data not previously published. Details of the linkage groups mentioned in Table 3 are given by Hutt and Lamoreux (1940). Both *D* and *Po* are independent of one or more genes in the first three groups listed. The fourth one, containing the mutations silky and flightless, has been tested with *D* and *M*, but data of the present writers on the possible linkage of *Fl* and *Po* were not satisfactory because *Po* was suppressed in those matings. From this summary it seems probable that the genes *D*, *M* and *Po* comprise a separate linkage group, the fifth autosomal one to be found in the fowl. Seven genes not previously assigned to any linkage group are also shown to be independent of both *D* and *Po*.

Summary

Polydactyly was found to be linked with multiple spurs and with duplex comb. The arrangement of these genes and the approximate crossover distances separating them are: *D* 28 *M* 33 *Po*. The amount of crossing over measured between *D* and *Po* was 42 per cent. and there were 9.3 per cent. double cross overs.

These determinations were made in backcross populations showing a normal 1 : 1 ratio of polydactylous to four-toed birds. It is shown that interpretation of linkage tests with *Po* is difficult when, as frequently happens, inhibiting genes prevent the manifestation of polydactyly in heterozygotes. Genes suppressing this mutation seem also to suppress multiple spurs.

A summary is given of published evidence and new data showing that *D* and *Po*, at opposite extremes of this link-

age group, are apparently independent of four other autosomal linkage groups and of seven other genes with which both have been tested.

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REVIEWS AND COMMENTS

EDITED BY CARL L. HUBBS

In this section reviews and notices are given of current publications on general biology and of specialized works which have an important bearing in this general field. Emphasis is given to books and major articles which fall within the special scope of The American Naturalist, in that they deal with the factors of organic evolution.

Reviews and Comments are meant to include also such general discussions, reports, news items and announcements as may be of wide interest to students of evolution. Except as otherwise indicated, all items are prepared by the Section Editor, Dr. Carl L. Hubbs, University of Michigan, Ann Arbor, Michigan. All opinions are those of the reviewer.

Race, Reason & Rubbish A Primer of Race Biology. By Gunnar Dahlberg. Translated from the Swedish by Lancelot Hogben. New York: Columbia University Press, 1942: 1–240, figs. 1–44. $2.25.

While serving as director of the State Institute of Human Genetics in the University of Uppsala, Gunnar Dahlberg prepared for the Swedish public a rational and studious discussion of one of the hot questions of the day. Lancelot Hogben, feeling that a wider reading of the Dahlberg product would be good medicine for a sick world, has translated the book into what he calls the Anglo-American language.

Dahlberg is convinced that the popular ideas that are rampant to-day regarding human races are largely erroneous, because they are based on prejudice and politics rather than on scientific principles. Consequently he devotes much of his book to a popular and generally excellent presentation of the facts and interpretations of modern genetics. This treatment is marred by some confusing errors in the calculations of Mendelian ratios (on pp. 80, 142 and 144). These slips are attributable, I suppose, to the difficult conditions under which the book was seen through the press.

The basic principles of genetics are then applied to human biology. It is held that through panmixia the ratios of genes in human populations tend to remain con-

stant, except as they are altered by mutation, selection, inbreeding, assortive mating and isolation. The last-named factor, unnecessarily called "the isolate effect," is held to comprise one of the two fundamental questions of human genetics, ranking in significance with selection arising from different fertility in social groups. The recent enlargement of the "isolates" (areas or units of effective breeding populations), through migration to towns, industrialization and increased communication, is regarded as a potent eugenic force. The assigned reason is that defective genes tend to increase in small breeding units, and to decrease in larger "isolates."

It is held that selection, as through sterilization, can not be effective in significantly reducing the incidence of recessive defects, because such characters are rare. Since persons showing the defects are few, a relatively high ratio of unaffected heterozygotes would continue to carry the defects and lead to the production of defective homozygotes in scarcely reduced numbers, even though most of the unfortunate should be prevented by law from reproducing.

Dahlberg's contributions to the understanding of race problems in man are significant and demand attention. Some of his views, however, seem inconsistent, and perhaps too strongly flavored with the social as opposed to the biological viewpoint. Racial differences in anything but outward characters are treated as improbable, because not yet proved to the author's complete satisfaction by objective research. Individual variations, particularly in the European groups, are taken as evidence against race concepts. Dahlberg strongly discounts the generally accepted idea of ethnologists that varying and mixed characteristics in human populations are traceable to the interbreeding of races that were previously more distinct. In this view he seems to have swung as far to one extreme as some anthropologists have gone to the other extreme in assigning members of a single interbreeding population to distinct racial types.

The Vertebrate Eye and Its Adaptive Radiation. By GORDON LYNN WALLS. Bloomfield Hills, Mich.: Cranbrook Institute of Science, Bull. 19, 1942: i–xiv, 1–785, 2 pls., figs. 1–197. $6.50.

AN active and accomplished researcher seldom takes time off to prepare a comprehensive treatise on his specialty. When he does he is apt to enter into great detail, to emphasize controversial points, and of course to think and write in so technical a manner, that his product is of use only to his fellow specialists (who will likely be in least need of such a resumé). Or he may underestimate his readers' capacities and delete technical information so largely that his book is not truly informative.

Walls set his course between these two extremes—a very difficult bit of navigation. Scientists will long for more documentation, for more thorough treatment of alternative theories, often for more detail; non-scientists will be repelled from time to time by technicalities, even though the job of sugar coating has been very skillful. The compromise in treatment and verbage has in general been so masterful, however, that the book will serve very well the needs of a wide range of readers. Oculists, medical practitioners, general zoologists, psychologists, and wideawake persons in many non-biological fields, now have available a full and understandable account of how all the vertebrates see, of what they see, and of how the seeing as they do fits them for the life they lead. The varying anatomy, function, phylogeny, and adaptation of the vertebrate eye are all dealt with in this large and handsome volume. Never before has so much light been shed at one time on an animal organ.

This is a tremendously interesting and valuable book. It deserves a place on thousands of library shelves.

Sex Hormones. Edited by F. C. KOCH AND PHILIP E. SMITH. Biological Symposia, Vol. IX. Lancaster, Pa.: The Jaques Cattell Press, 1942: i–x, 1–146, 46 figs. $2.50.

IN the ninth volume of BIOLOGICAL SYMPOSIA there are published the contributions to the Symposium on Sex

Hormones, which was one of the features of the Fiftieth Anniversary Celebration of the University of Chicago, and to the Symposium on Hormonal Factors in Sex Inversion, which was held at the April, 1942, meeting of the American Association of Anatomists.

The first series treats the actions and metabolism of the sex hormones. The foreword is by FRANK R. LILLIE, and the four papers are: "The Comparative Biology of Testicular and Ovarian Hormones," by CARL R. MOORE; "The Comparative Metabolic Influences of the Testicular and Ovarian Hormones," by A. T. KENYON; "The Metabolism of Estrogens," by EDWARD A. DOISY, and "The Excretion and Metabolism of Male Sex Hormones in Health and Disease," by F. C. KOCH. Together these contribute an important contribution in one of the most active branches of current biological progress.

The second series of symposia deal with "Hormonal Factors in the Inversion of Sex." The papers are by C. H. DANFORTH R. R. HUMPHREY, R. R. GREENE, and R. K. BURNS, JR., and deal with sex-inversion experiments, respectively on birds, amphibians, the rat, and the opossum. An understanding of the basic biology of sex is markedly advanced by these studies.

The Crayfishes of Florida. By HORTON H. HOBBS, JR. University of Florida Publication, Biol. Sci. Ser., 3, 1942: i–v, 1–179, pls. 1–24, maps 1–11, figs. a–c. $2.25 plus postage.

THE University of Florida has become an outstanding center of natural history research. Stimulated by their location in a region where the fauna is extremely rich and varied, yet very poorly known, the Florida zoologists are engaged in thorough studies of Southeastern animals. Working with rare enthusiasm and energy they are constantly making discoveries of great taxonomic, distributional, ecological and evolutionary significance. Among the most notable of these studies is that of Hobbs, on the crayfishes of Florida.

This student has found an amazingly rich cambarid

fauna in Florida—42 species and subspecies. In defining these forms Hobbs has been led to a revision of the classification of the Cambarinae. Instead of the single genus *Cambarus* he recognizes six genera, including the remarkable new blind genus, *Troglocambarus*.

Because Florida has so recently emerged from the sea, Hobbs has been able to trace plausible routes by which the several speciating lines may have entered the state. As a result of thorough field work, present distributions and habitats have been well defined and correlated. Isolation is indicated as an important speciational factor, particularly in the varied topography and separated stream systems of the Florida panhandle. Some of the characters are held to be of adaptive significance. Speciation has apparently been rapid as well as extensive, both in open waters and in caves. Though the author does not stress this point we seem to have here a splendid example of the theory that evolution is ordinarily a slow process, because it is retarded by the biotic saturation of old lands and waters. When this repressive force is weakened, speciation becomes free and rapid. When almost totally unoccupied habitats become available, as they did when the rift lakes of Africa were born, and when Florida rose from the sea, evolution may become explosive. Nature abhors a vacuum.

Studies on the Origin and Early Evolution of Paired Fins and Limbs. By WILLIAM K. GREGORY AND HENRY C. RAVEN. Ann. N. Y. Acad. Sci., 42, 1941: 273–360, figs. 1–34, pls. 1–5, $1.00.

THESE new attacks on old problems bear on some of the fundamental concepts of phylogeny. The authors counter a generally accepted tenet of phylogeny, that highly specialized groups are not the stuff out of which major phyletic lines are born. The ostracoderms—weird mailed fishes of the Paleozoic—are regarded as the ancestral stock of fish evolution; not as offshoots which were preserved by reason of their specialized exoskeleton, while contemporary and less specialized kinds, theoretically

more logical as ancestral stocks, died without leaving a known trace. The external armor is held by Gregory and Raven to have become reduced and modified in the other fish groups to form not only the bones of the skull but also the skeleton of the appendages. The views of these authors are dependent on certain assumed phyletic relationships of the major fish groups. But since these main lines sprang into existence almost simultaneously soon after the origin of the vertebrates, and since the record is highly incomplete, one may with at least equal plausibility assume other lineages, which would call for radically different concepts of comparative anatomy and evolution. The simple skeleton of the fold-like paired fins of the primitive Actinopteri, as exemplified by *Polyodon,* suggest what I still regard as the most plausible theory as to the mode of origin of the limbs, a theory that is compatible with the idea that generalized animals alone are capable of embarking on major evolutionary journeys.

The main portion of these studies deals with another problem, the transformation of the paired fin of the Pisces into the pentadactylate limbs of the Tetrapoda. Here the authors come out of the quagmires (as the ancestors of the tetrapods did) to tread on firmer ground; to display more convincingly their mastery of paleontology, anatomy and taxonomy; and to contribute more securely to our ideas on the evolution of the vertebrates.

ARTICLES ON SPECIATION AND EVOLUTION

Adaptive Modifications for Tree-trunk Foraging in Birds. By FRANK RICHARDSON. Univ. Calif. Publ. Zool., 46, 1942: 317–368, pls. 23–24, figs. 1–16. $0.75.—Many parallel and some compensatory adaptations are demonstrated to fit certain birds for the tree-trunk foraging by which they obtain their food. The adaptations are regarded as parallel instead of convergent, because they involve homologous rather than nonhomologous structures. This criterion, also advocated by Abel, is preferred by the author over the more common one that is based on degree of relationship. There are obvious advantages in each point of view, and the issue is confused by applying the same terms to

distinct concepts. I would rather contrast homologous and non-homologous adaptations, as well as distinguishing between parallel and convergent ones. I agree with Richardson's view that "natural selection seems to be confirmed by the very existence of structural adaptations," but regard as indefensible his restriction of the adaptation concept to "structural modifications mechanically suited to the use to which they are put." To delete habits from such concepts as adaptation and homology is to revert to the age when morphology was unduly emphasized, and hinders clear evolutionary thinking.

The Osteology and Myology of the California River Otter. By EDNA M. FISHER. Stanford University: Stanford University Press, 1942: i–vi, 1–66, figs. 1–37 (offset printing). $1.50.—This study was undertaken as an accessory to the author's researches on the aquatic adaptations of the sea otter. The river otter shows a lesser degree of modification for life in water. In order to permit the desired comparisons, the anatomy of the river otter had to be worked out in fine detail.

Geographic Variation in Garter Snakes of the Species Thamnophis sirtalis in the Pacific Coast Region of North America. By HENRY S. FITCH. Am. Midland Nat., 26, 1941: 570–592, figs. 1–3.—This report is a sequel to the same author's outstanding study of speciation in *Thamnophis ordinoides*, which was reviewed in these columns (AM. NAT., 75, 1941: 384–386). The species *sirtalis* proves to be the more uniform in habitat preference and in characters. It shows some geographical variation in the ventral and caudal scutes, but the systematic significance of these variations is discounted. Regional variations in coloration, which form a different and a more consistent geographic pattern, are used to diagnose four western subspecies. There are marked local variations within these subspecies, and sharp distinctions occur on the two sides of barriers. The local variations in *sirtalis* often follow those in *ordinoides*. In both species the adult size and the number of scutes decreases toward the north, toward higher elevations and toward the coast. There are parallelisms too in coloration, and some of the local races of the two species that live together are remarkably alike. The several color types appear to be concealing in their local environments, and are therefore held to be of probable adaptive significance.

Some Aspects of Evolutionary Theory. By GEORGE M. ROBERTSON. Fort Hays Kansas State College Studies, Gen. Ser., 4 (Sci. Ser. 1), 1942: 113–167.—The taxonomy and phylogeny of the lower vertebrates is the primary subject of these essays. There is offered an outline classification of the Chordata, reflecting the discoveries and opinions of paleontologists and embodying some original ideas. Consideration is also given to the bearing of certain paleontological and embryological data on some problems of homology. Principles of phylogeny are discussed and the conclusion reached that phylogeny can become a science only with the development of tested generalizations.

Statistical Genetics and Evolution. By SEWALL WRIGHT. Bull. Am. Math. Soc. 48, 1942: 223–46, figs. 1–10.—A treatment of the statistical consequences of the evolution theory, with particular emphasis on changing gene frequencies.

SHORTER ARTICLES AND DISCUSSION

FURTHER NOTES ON DIFFERENTIAL SELECTION OF VARIANT JUVENILE SNAKES

A comparison of juvenile and adult populations of the smooth green snake, *Opheodrys vernalis* (Harlan), supports the conclusions of E. R. Dunn (1942) as to differential selection in juvenile snakes (Inger, 1942). A similar study of *Thamnophis radix* Baird and Girard, using 157 adults and 160 juveniles, affords further information on this subject. The specimens examined are from the adjacent Cook, Lake, and McHenry Counties, Illinois, and Walworth County, Wisconsin, and are contained in the collections of the Field Museum of Natural History. The juvenile specimens are 200 mm or less in length; of the adults none is smaller than 400 mm, which is approximately the size of the smallest reproductively mature individual (Cieslak, 1938). I wish to express my gratitude to Messrs. Karl P. Schmidt, and Clifford H. Pope, and Dr. Carl L. Hubbs for their valued criticism and aid and to the Field Museum of Natural History for the use of its laboratories and collections. I am also indebted to Dr. Sewall Wright, of the University of Chicago, for his comments on the data contained in this paper.

A feature of the comparison between the two age groups shown in Table 1 lies in the difference of the means of certain scutellation characters. The difference in the means between the juvenile and adult series is significant in the ventrals and caudals only. It is interesting to note that there is uniformity in the differences which are significant.

The juveniles show a larger coefficient of variability than the adults in every character examined, indicating a greater variability of the juvenile population, from which a differential selection is inferred. In the case of the ventrals, the reduction of the coefficient of variability is not brought about through the loss of individuals from both extremes of the observed range of the character, as would be expected. The reduction is due instead primarily to the elimination of individuals from the lower extreme. The same trend is apparent in the caudals. This is illustrated in Figures 1 and 2.

The explanation of such differential selection is not clear. The negative selection of anomalies may well be due to their associa-

TABLE 1

COMPARISON OF SCUTELLATION OF JUVENILES AND ADULTS OF THAMNOPHIS RADIX

Character	Sex	Age	Mean	Difference of means	Coefficient of variability	Number examined[1]
Ventrals	♂	adult	156.71	2.15 ± .545	2.02 per cent.	85
	♂	juvenile	154.56		2.35 " "	74
	♀	adult	151.05	2.18 ± .565	2.13 " "	72
	♀	juvenile	148.87		2.61 " "	86
Caudals	♂	adult	73.75	1.78 ± .603	4.70 " "	65
	♂	juvenile	71.97		4.95 " "	71
	♀	adult	64.80	2.42 ± .540	4.35 " "	56
	♀	juvenile	62.38		6.36 " "	84
Infralabials	♂ + ♀	adult	9.80	.02 ± .03	4.30 " "	308
	♂ + ♀	juvenile	9.78		5.25 " "	318
Supralabials	♂ + ♀	adult	7.19	.....	5.80 " "	313
	♂ + ♀	juvenile	7.19		6.56 " "	318
Postoculars	♂ + ♀	adult	2.98	.....	4.62 " "	314
	♂ + ♀	juvenile	2.98		4.95 " "	318
Temporals (2nd row)	♂ + ♀	adult	2.18	.02 ± .03	19.4 " "	312
	♂ + ♀	juvenile	2.16		20.7 " "	320

[1] Number refers to specimens in ventrals and caudals. Number refers to sides of heads in other characters.

tion with other, less evident, physiological deficiencies. By way of further speculation, it may be pointed out that the evolution of snakes appears to exhibit a trend toward an increase in flexibility—a flexibility necessarily accompanied by an increase in the strength of the spinal column. To an animal without appendages, flexibility of body is obviously of great survival value. Increase in strength of the vertebral column (over that of lizards)

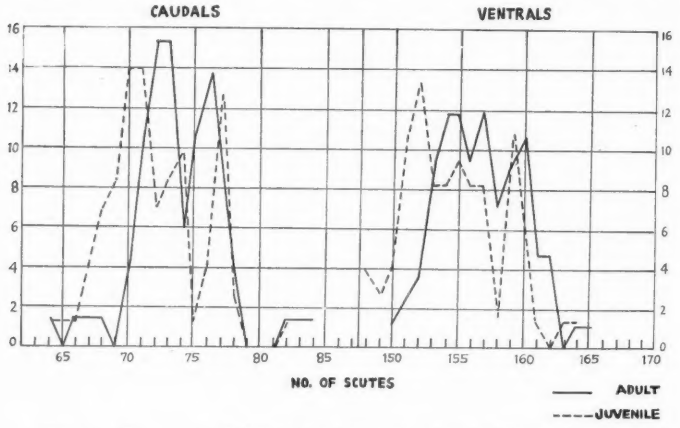

FIG. 1. Frequency distribution (percentage) of abdominal scutes of males of *Thamnophis radix*.

is produced by additional articulations between the vertebrae. These additional zygapophyses, the zygosphenes and zygantra, have added so much to the rigidity of the articulation between vertebrae that any bend in a snake's body usually involves at least ten vertebrae. A net increase in flexibility can come about therefore only by adding to the number of vertebrae.

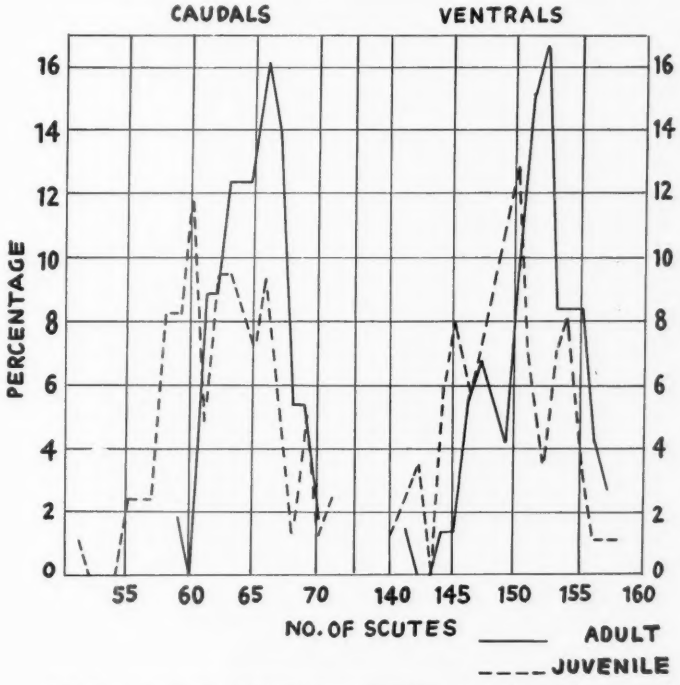

FIG. 2. Frequency distribution (percentage) of abdominal scutes of females of *Thamnophis radix*.

The number of ventrals (and caudals) is a measure of the number of vertebrae. A reduction in the number of vertebrae (and the correlated reduction in ventrals) may be thought to have negative survival value because it would decrease flexibility, and natural selection would thus tend to eliminate individuals showing such a reduction. That this is true may be seen from the data in Figs. 1 and 2.

Other factors must be supposed to limit the maximum of vertebrae. The difference between the mean of the ventrals (and

caudals) of the juveniles and the mean in the adults obviously does not indicate a continuing increase in the number of ventrals in this species; for *Thamnophis radix* with a mean of ventrals of 154 may be supposed to have maintained about this number for several millennia. If a difference as large as shown, existing between two generations, were continued from generation to generation, this species would have upward of 1,000 ventrals in the space of two centuries. The change in the mean number of ventrals (and caudals) from juvenile to adult therefore does not represent an evolutionary advance or even a genetic change, but a selection mechanism for maintaining the number of ventrals at the norm established in the evolution of the species.

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A BASIS FOR OSTENSIBLE REVERSAL OF EVOLUTION

THE non-reversibility of evolution is a biological law which is indisputably valid as a generality, yet occasionally there appears a phylogenetic sequence which seems to result from a reversion to an ancestral condition. An example, to mention but one, is the occurrence of teeth in the lower jaw of the amphibian genus *Amphignathodon*. This condition is not found in any of the other genera of the Hylidae, a relatively advanced family, nor in any other anuran. In all the phylogeny of the anura, no teeth are known until we reach this specialized family. Dr. Norman Hartweg has suggested that such exceptions may be special cases of the law of non-reversibility rather than contradictions, inasmuch as these reversions do not necessarily entail a re-evolution of structures completely lost. We can think of a reversion as a phyletic atavism—the evolutionary unmasking of long-hidden primitive characteristics. Conceivably all the genetic and developmental requirements for an atavistic character might be pres-

ent, but suppressed in a specialized organism. There is good experimental evidence for such a hypothesis.

Suppressor Genes

If we conceive of an apparent reversal of evolution as due to a reappearance of suppressed genetic potencies, we must first seek known cases of the suppression of one gene by another. This is not the same as the dominant-recessive relationship between alleles, but is suppression of a gene in one locus by a gene in another. The term epistasis is applied when one gene has an effect of its own and also suppresses another which is not its allele. Perusal of any genetics text will provide numerous examples. For instance, in *Drosophila*, the factor *su* in chromosomes III will suppress purple eye color, for which the genes are in chromosomes II (Bridges, 1932). Thus the genotype *pr pr* II with + + III produces purple eye, as the normal wild-type (+) genes in chromosome III do not interfere. Introduce the suppressor, *pr pr* II with *su su* III, and wild-type eyes result. By such a mechanism, a race of purple-eyed *Drosophila* might revert to wild-type without any change in the purple factor itself, merely as a result of the introduction of the suppressor. Of a somewhat different type is the suppression of sepia eye by the white eye factor. A fly homozygous for both is white-eyed. Replacement of one *w* gene by w^+ allows the sepia to show. Epistasis and suppression occur in all types of organisms. The albino factor in mice hides any genetic color pattern present, and gray prevents the expression of black. In fowls there is a color suppressor, and another in squashes. The influence may be partial or qualitative, in which case the gene is known as a modifier, and a whole series of modifiers may be present, producing a graded series. Mice homozygous for spotted coat may vary from almost solid color to almost complete lack of color, depending upon the number of modifiers present. The sepia mentioned above and certain other eye color factors can be considered modifiers of the wild allele of white.

An instance which might easily be termed reversal of evolution by one not sworn to uphold the paleontological postulates is polydactyly in the guinea pig (Wright, 1935). Selection produced heterozygous five-fingered, four-toed guinea pigs from the four-fingered, three-toed wild type. This is not a case of a single-factor suppressor, but it is genetically controlled atavism in that favorite of the paleontologist—digit pattern.

Clearly there is an abundance of genetic evidence favoring the possibility that a gene may be carried through a number of generations without affecting the phenotype, or having only a partial effect, and subsequently recover its full phenotypic expression. How long such a suppressed condition might persist, having no selective value, may presumably be judged from the persistence of vestigial organs which are neither helpful nor harmful.

EVIDENCE FROM EXPERIMENTAL EMBRYOLOGY

It might be argued that in taking evidence from the action of single genes, we are over-simplifying the problem. A taxonomically important modification involves a whole organism, not a single-factor character. It must be kept in mind, however, that ontogeny is a synergy of catenated phenomena, and failure of a single process can alter all subsequent events. The giant-lethal mutation in *Drosophila* is a single-gene effect inhibiting the pupation hormone (Hadorn, 1937). As a result, the larva continues to grow and never pupates. Injection of the hormone allows the whole complicated process of pupation to continue. Action of a single gene alters the entire life of the individual. Experimental embryology has revealed dependent sequences to be the rule. As an example could be taken the gill, which depends upon the presence of ectoderm over the mesoderm gill field. This ectoderm normally forms gill, but if removed from the influence of the gill field before its fate is determined, it does not form gill. Reciprocally, flank ectoderm will form gill if transplanted to the gill field. Without altering the genetic constitution of that ectoderm, we can call forth or suppress its gill-forming potencies. Similarly, the gill field is determined by preceding organizers, and so on, back to the primary organizer. It is not too much to postulate that somewhere in this chain of events a single gene could act to prevent formation of gills.

A temporary suppression of gills occurs in the *Desmognathus fuscus* larva while it remains on land (*cf.* Noble, 1931). In *Breviceps* and some other microhylid toads, gills are entirely lacking. Here experimental embryology opens the theory to experimental attack. If it is the ectoderm in *Breviceps* that lacks gill-forming potencies, transplantation to the gill region of a gilled amphibian should reveal that lack. Reciprocal transplants would determine whether the inhibition acted upon the mesoderm gill field, or upon the ectoderm, or both. Other larval organs are open to the same approach.

Life Cycles Reveal Gene Suppression

We can not study the physiology of gene suppression in an evolutionary series, but some life cycles reveal a comparable phenomenon within a shorter span of time. One such is the parthenogenetic-bisexual cycle that occurs in some insects, rotifers and cladocerans (Shull, 1925). A wingless female aphid, coming from several generations of parthenogenetic females, will give birth parthenogenetically to a female that develops wings. The chromosome numbers are the same and there has been no opportunity for the introduction of new genic material, so we can only conclude that the wing-producing functions were suppressed. Moreover, a series of parthenogenetic females will end with females which produce males or females capable of sexual reproduction. Clearly whole complexes of genetic potentialities can survive a number of generations without phenotypic expression, to reappear at a later time, all within a normal life cycle.

Conclusion

There are already known to exist all the processes which would be requisite for evolutionary atavism. Genic behavior and embryological process supply the necessary mechanisms, and variable life cycles show that such masking and unmasking occur naturally. In the fossil record, such release of masked characteristics would have the appearance of a reversal of evolution, whereas it actually would be a re-emergence of unaltered ancestral characteristics. It would not be reversal in that the structures and functions were not evolved anew. With regard to the interpretation of phylogeny, however, it would serve essentially the same as a reversal.

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EFFECTS OF SILVER NITRATE ON THE PIGMENTATION OF DROSOPHILA

RAPOPORT (1939) has published preliminary data on the production of phenotypic modifications in *Drosophila melanogaster*, by raising the larvae on food containing sublethal doses of certain chemical compounds. Some of these modifications appeared to resemble certain mutant types known in the same species; these modifications are considered phenocopies. In particular, treatments with silver lactate and some other (not specifically named) silver compounds produce, according to Rapoport, "sharp reduction of the body color, perfectly resembling extreme alleles of the mutant yellow." The observations described below confirm and extend those of Rapoport on the effects of treatments with silver salts.

The concentration of silver nitrate which is sublethal for Drosophila larvae has been determined. Stender jars with an inside diameter of 2 inches were provided with 10 cc of the regular cornmeal-molasses-agar food to which definite amounts of a 10 per cent. solution of silver nitrate had been added during the preparation. Five series of jars were prepared containing 0.1, 0.05, 0.01, 0.005 and 0.001 grams of silver nitrate per 10 cc of food, respectively. Flies were allowed to oviposit on spoons with the regular silver-free food. Known numbers of freshly hatched larvae, no more than two hours old, were transferred to the experimental jars and allowed to develop to the adult stage. The jars with the silver-treated food were, as far as possible, protected from light. In the jars with 0.1 and 0.05 grams of the silver salt all the larvae died. The concentration of 0.01 grams of silver nitrate per 10 cc of food was found to permit enough larvae to survive. This concentration was used as the standard in all subsequent experiments.

The wild type of *Drosophila melanogaster* raised in jars with the standard concentration of $AgNO_3$, as well as in jars with 0.005 grams per 10 cc, showed a very pale body color. The flies hatched in the jars with 0.001 grams of $AgNO_3$ per 10 cc were apparently normal. The body color in the modified flies is a pale yellowish gray, distinctly different, contrary to the statement of Rapoport, from that in any allele of yellow with which the writer is familiar. It resembles most closely that in the sex-linked recessive mutant straw, but is even somewhat paler and grayer than straw. Not only the integument proper but also the

bristles and the microchaetae on the fly's body are paler than normal. The dark bands on the abdominal segments in both sexes are still visible but are much less distinct than normal. The chitin is delicate and gives the impression of being softer or more elastic than normal. If the silver-treated flies are a phenocopy of any known mutant, this mutant is straw and certainly not yellow or "silver."

Aside from the wild type, certain mutants of *Drosophila melanogaster* were also tried on the standard silver-containing food. The ebony mutant becomes strikingly paler, but by no means as pale as the treated wild type. The treated ebony is about intermediate in coloration between the untreated wild-type and the untreated mutant sooty, which is a lighter allele of ebony; the fly is, however, suffused gray, showing no distinct trident pattern on the thorax and no distinct dark bands on the abdomen. The treated black-speck becomes lighter than the untreated speck without black. The mutant yellow raised on the standard silver-containing food is paler than the untreated controls, but the difference is by no means as striking as that between the treated and untreated wild-type or ebony flies. Finally, the mutant straw changes little if at all as a result of silver treatment. Two other species, which are normally much darker in the body color than *D. melanogaster*, namely *D. pseudoobscura* and *D. virilis*, have been tried. When raised on the standard silver-containing medium they become very much paler than the untreated controls, but they preserve a smoky brownish gray coloration which is much darker than that in the straw mutant of *D. melanogaster*.

Aside from the changes in pigmentation of the integument, all the treated flies of the three species examined show also changes in the color of the pericardial cells and of the Malpighian vessels. The pericardial cells in the untreated flies contain little or no pigment; in the treated ones these cells are of a dark brownish red color. Two rows of these cells are clearly visible through the body wall of the dorsal surface in the treated flies. It may be desirable to point out specifically that the red pericardial cells appear also in treated straw flies in which little change of the body coloration is perceptible. The red coloration of the pericardial cells is clearly present not only in the adult flies but also in the larvae developing on the silver-containing food. The normal greenish pigment of the cells in the Malpighian vessels is changed to a reddish brown, in the adults as well as in the larvae.

An attempt has been made to determine whether or not there is a period in the larval development during which the silver treatment produces its effects on the coloration of the adult fly. Freshly hatched wild-type larvae of *D. melanogaster* were collected at one-hour intervals and placed in jars with silver-free food. From time to time a group of larvae of known age were transferred to the standard silver-containing food, allowed to remain there for 24 hours, and returned back to the normal food to complete their development. The larvae which have been kept on silver-containing food from hatching from the eggs to the age of 24 hours gave rise to adults with a straw body color, pinkish pericardial cells and greenish Malpighian vessels. Larvae treated at any age older than 24 hours give straw-colored adults with deep orange pericardial cells and brownish Malpighian vessels. There is, thus, no pronounced sensitive period during which the effects of the silver treatment would be produced.

The physiological mechanisms intervening between the ingestion of the silver salt by the larvae and the appearance of the abnormal colorations in the adult are unclear. It is, however, doubtful whether the silver-treated flies may be justly called phenocopies of straw or of any other known mutant. Indeed, aside from a slight difference in the coloration of the integument, the straw flies do not show the changes in the pericardial cells and in the Malpighian vessels which are so characteristic of the silver-treated flies.

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